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he Peru-Chile Undercurrent[1]

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ABSTRACT

The presence of a southward-moving undercurrent along the west coast of South America, lerlying the Peru Current at depths of several hundred meters, has been established by ct measurements with parachute drogues, by calculations of geostrophic motion, and analysis of the distributions of salinity and dissolved oxygen. The undercurrent extends n northern Peru to at least 41°S. Speeds of 4–10 cm/sec were measured off Peru and thern Chile; the geostrophic transport appears to decrease downstream, from 21×10^6 at to about 3×10^6 m3/sec at 15°S. At its coastal edge the flow parallels the edge of the tinental shelf. South of 15°S, waters of the undercurrent are characterized by relatively n salinity and temperature as well as low oxygen content.

The surface currents which flow equatorward along the eastern margins the major ocean basins are relatively well known. They include the Cali- nia and Peru currents in the Pacific Ocean and the Canary and Benguela rents in the Atlantic Ocean. But little, if anything, is known about the

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subsurface circulation in these regions, although there are some indicatio of poleward coastal currents several hundred meters beneath the equatorwa surface flow.

For example, a common feature of the density distribution on cross-strea profiles off such coasts is a deepening of the isopycnals shoreward below t ascending isopycnals characteristic of coastal upwelling (Wooster and Rei in press). A similar deepening (or trough) is also found along the equator association with the Equatorial Undercurrent or Cromwell Current (Knau 1960). In the coastal region geostrophic computations based on this densi distribution indicate the presence of a subsurface countercurrent, or unde current.

The similarity in distributions of mass and circulation in the equatorial a eastern boundary coast regions has been noted by Yoshida and collaborato (Yoshida, 1955; Yoshida and Tsuchiya, 1957; Yoshida 1958, 1959), w have made a theoretical investigation of the boundary phenomena. But un now no one has actually measured a coastal undercurrent and attempted relate such measurements to other physical and chemical observations ma in the vicinity at the same time.

In a recent paper Wooster and Reid (in press) discussed the evidence f coastal undercurrents on the eastern boundary coasts of California, Peru a Chile, northwest and southwest Africa. The scarcity of data suggested t desirability of including direct current measurements in the scientific progra of the STEP-I Expedition of the University of California's Scripps Institut of Oceanography. The present paper is based primarily on observations this expedition aboard R/V Horizon off the west coast of South Ameri during October 1960.

Observations and Methods. The track and station arrangement of t STEP-I Expedition are shown in Figs. 1 and 2. Observations along the pr files consisted of oceanographic stations at 40–100 mile intervals with nume ous BT and surface salinity observations between stations. On most statio measurements were made between the surface and at least 1000 m, and some cases to depths of several kilometers. Station measurements includ temperature, salinity, dissolved oxygen, phosphate, silicate and nitrite, made conventional methods (salinities were determined with a conductivity-ty salinometer: Paquette, 1959).

South of Talara at six locations, measurements of surface and subsurfa currents were made with parachute drogues close to shore (Fig. 2) but beyo the edge of the continental shelf because the undercurrent was believed be a boundary phenomenon and because it was desired to use offshore islan as reference points for navigational control. The drogues, similar to th described by Jennings and Schwartzlose, 1960 (see Volkmann, *et al.*, 195 for discussion of the method), consisted of seven-meter diameter aviato

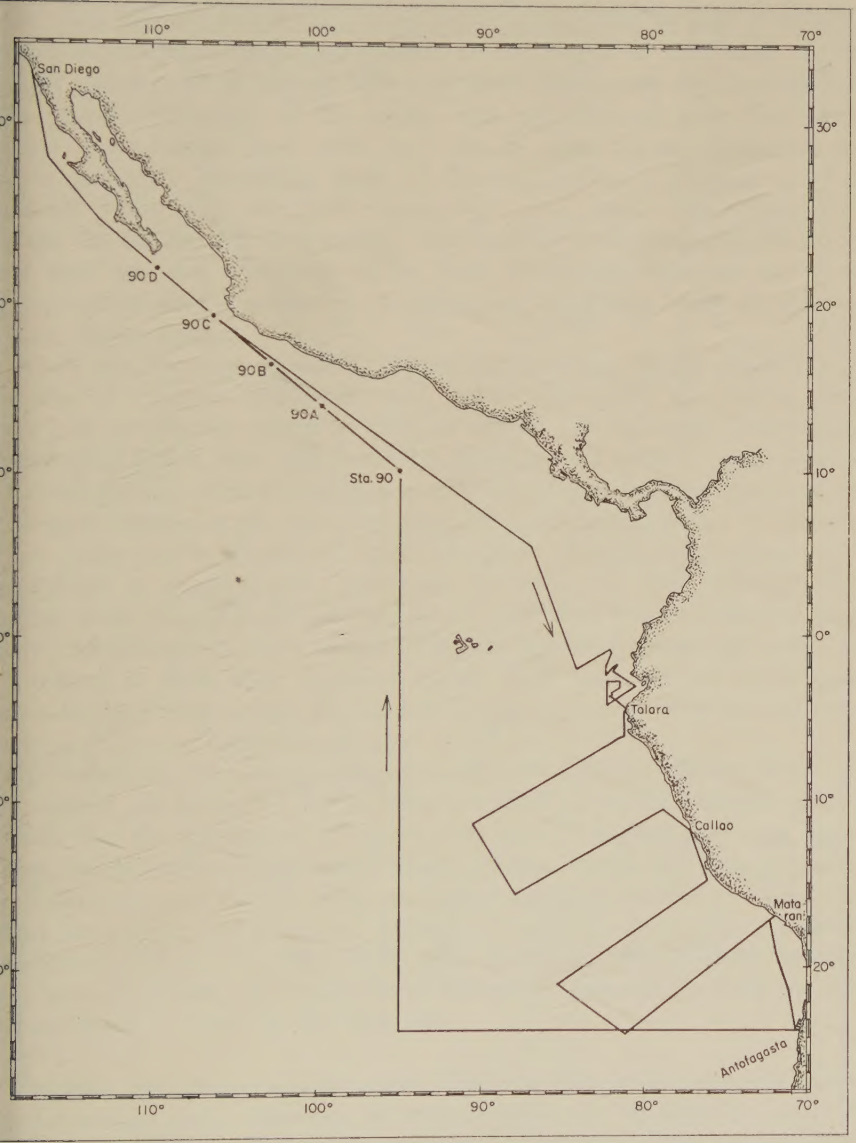


Figure 1. Track chart of STEP-I Expedition, 15 September to 14 December, 1960.

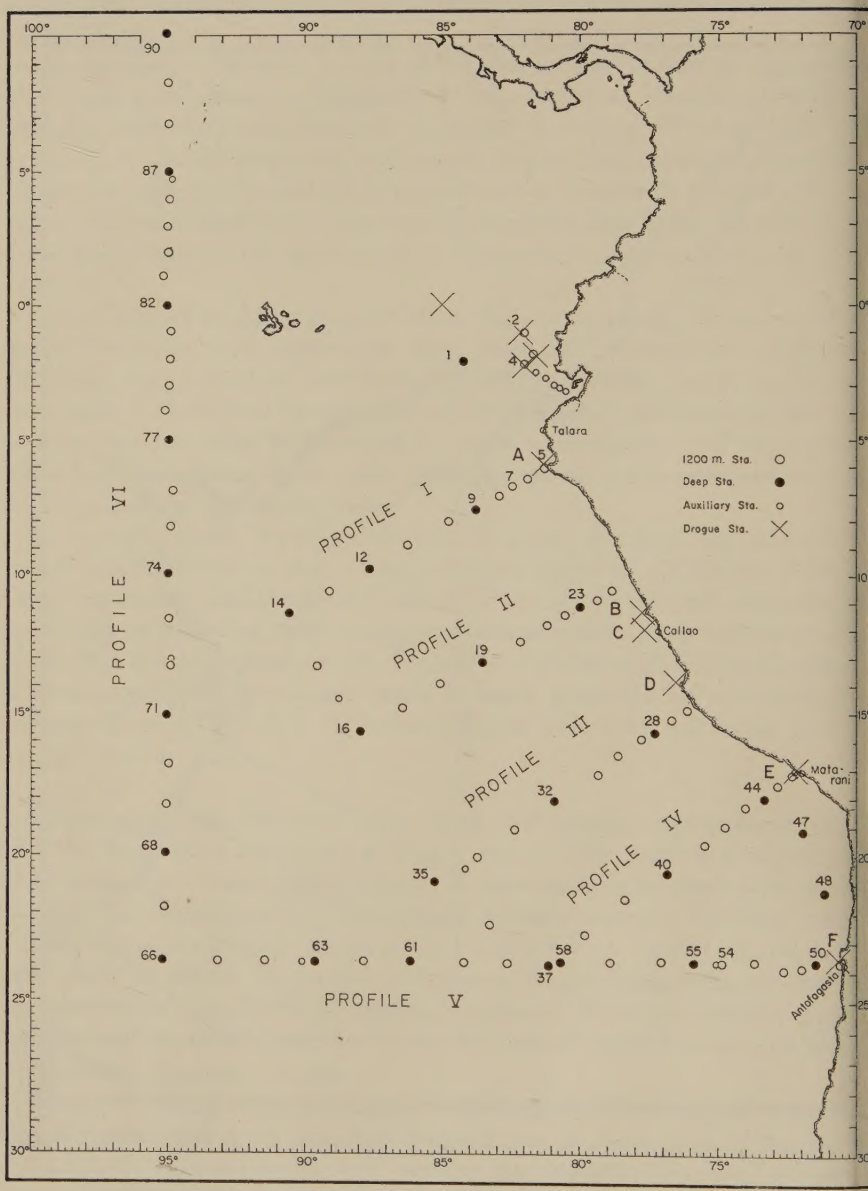


Figure 2. Station plan of STEP-I Expedition.

rachutes connected by wire to seven-meter bamboo poles suitably equipped serve as surface indicators of the drogue positions.

At each location two drogues were customarily placed at each of the following approximate depths as indicated by the length of wire used: 10 m, 00 or 150 m, 250 or 300 m. The true depth differs only slightly from the ire length (Volkmann, *et al.*, 1956). The parachutes at 10 m were connected to the surface floats with 7×7 aircraft cable, 2.4 mm diameter; for eeper parachutes, including those of the two reference drogues, 2.1 mm ameter music wire was used; connections were made with "Nicopress" eeves. On the southernmost station, combinations of aircraft cable and music ire were used due to shortage of the latter. Parachutes were sent unopened the desired depth, weights at the parachute apex then being dropped by time release device.

Along the Peruvian coast, numerous small steep islands are perched near e edge of a narrow continental shelf, and on four of the six stations, these ere used as reference points for position control. On the other two stations, here such islands were not available, parachutes at 1000 m were used as ference points. After a suitable interval following launching, the positions drogues relative to the reference point were determined with a U.S. Navy PS-5 radar and by occasional visual inspection. Ranges and bearings were termined at approximately half-hour intervals, the total tracking time rying from 18–43 hours. It was usually possible to track all drogues on a ation simultaneously, from ranges of several thousand meters (the radius of a return) to more than 14,000 m (under ideal wind and sea conditions). fter 12–24 hours, surface drogues had usually moved out of range and were andoned so that subsurface drogues would not be lost.

In analysizing these drogue measurements, the apparent relative motion of e drogues was corrected for the effect of wind and surface current on the rface float and for drag of the connecting wire. A discussion of drag forces ting on the various components of the drogue system has been presented Volkmann *et al.* (1956). The convenient graphical method of Brown (in ess) was used for computing the necessary corrections. As a rule, corrections r forces acting on the surface float were significant whereas those for wire ag were negligible. A discussion of the errors involved in these current easurements is given in the Appendix.

Evidence from drogue measurements. Pertinent information concerning the ogue stations and a summary of measurements are presented in Table I. he observed drogue trajectories, uncorrected for drag of the wire and surce apparatus, are shown in Figs. 3–8.

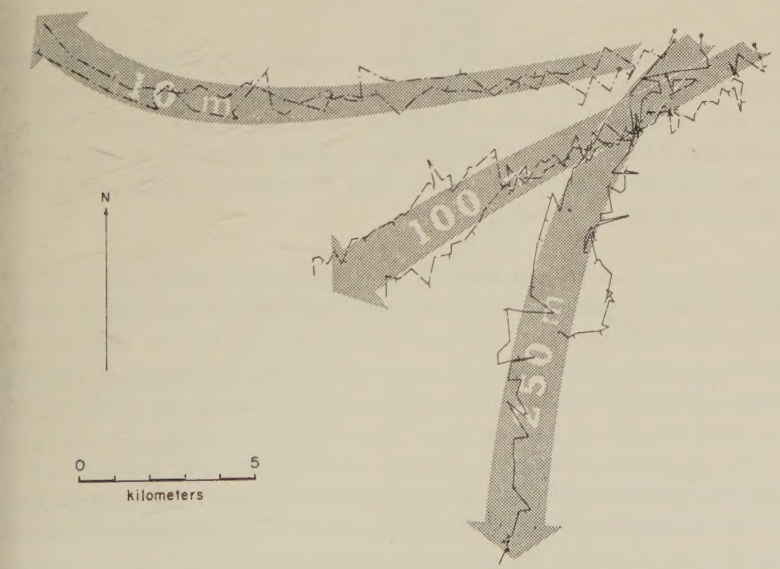
Corrected mean current vectors, estimated by comparing the first and last liable drogue positions, are shown superposed on charts of bottom topoaphy in Fig. 9.

TABLE I. Summary of Drogue Measurements.

Drogue Station	Station Depth (m)	Width of Cont. Shelf (km)	Distance Offshore (km)	Mean Wind cm/sec dir °T	Depth of drogue (m)	No. of drogues	Max. Time Observed (hours)	Observed Drift speed cm/sec	Observed Drift direction °T	Corrected Drift speed cm/sec	Corrected Drift direction °T
A	320	7	26	53/170	10	3	12.5	11.9 ± 3.5	292 ± 3	11	287
					150	2	28.1	3.1 ± 1.6	162 ± 15	5	160
					300	2	34.8	6.4 ± 1.3	151 ± 18	7	152
B	300	35	49	30/162	10	2	20.4	25.8 ± 0.9	272 ± 2	26	270
					100	2	35.2	9.8 ± 0.6	239 ± 4	8	225
					250	2	35.2	9.3 ± 1.8	199 ± 5	9	192
C	290	65	73	76/152	10	2	5.2	29.3 ± 2.3	306 ± 5	28	314
					100	2	18.1	9.5 ± 1.0	312 ± 8	5	310
					250	2	18.1	8.9 ± 0.7	318 ± 9	5	321
D	465	11	34	53/156	10	2	19.6	18.1 ± 0.4	352 ± 3	17	353
					100	2	6.2	2.6 ± 1.2	344 ± 38	0	–
					250	2	19.6	1.7 ± 1.0	321 ± 76	1	202
E	650	4	10	2/123	10	4	15.5	28.7 ± 3.2	308 ± 7	29	308
					100	2	34.0	17.5 ± 0.7	327 ± 2	15	331
					250	2	33.0	3.9 ± 1.8	141 ± 12	7	133
F	3135	3	27	37/168	10	2	22.7	19.3 ± 0.5	065 ± 2	19	067
					100	2	33.2	3.1 ± 0.3	151 ± 16	4	179
					250	2	33.2	4.8 ± 0.2	202 ± 5	6	211

A – 6 Oct. 1960, 5°57′S, 81°26′W; B – 19 Oct. 1960, 11°26′, 78°05′W; C – 25 Oct. 1960, 11°58′S, 77°49′W;
D – 27 Oct. 1960, 13°51′S, 76°43′W; E – 7 Nov. 1960, 17°0′S, 72°15′W; F – 12 Nov. 1960, 23°31′S, 70°53′ W.

igure 3. Uncorrected drogue trajectories, St. A (5°57′S, 81°26′W). Surface drogues indicated by dash-dot lines, intermediate drogues by dashed lines, and deep drogues by solid lines. Ticks along trajectory indicate 2-hour time intervals.



gure 4. Uncorrected drogue trajectories, St. B (11°26′S, 78°05′W); symbols as in Fig. 3.

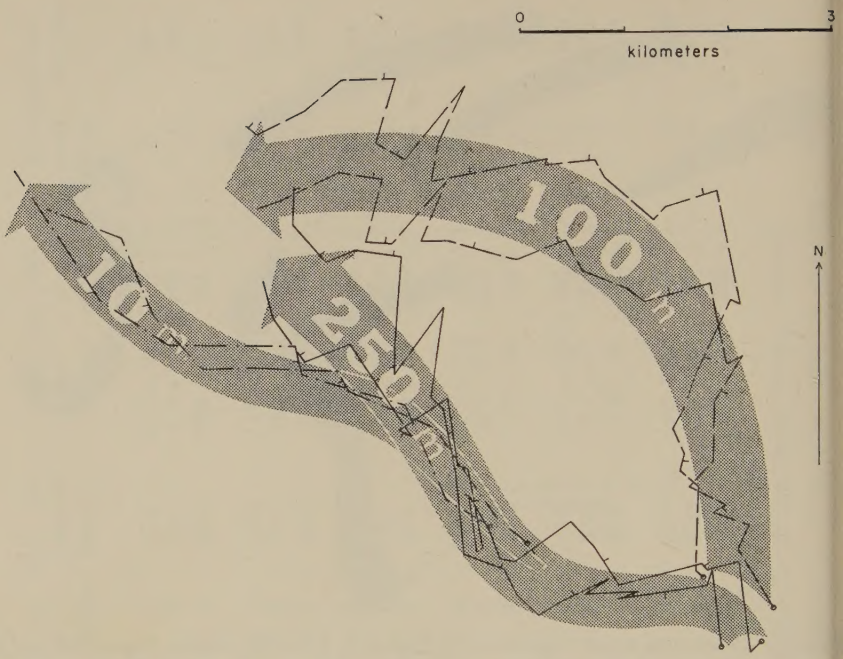


Figure 5. Uncorrected drogue trajectories, St. C (11°58′S, 77°49′W); symbols as in Fig. 3.

Within the time periods during which drogues were tracked, fluctuatio in speed and direction are evident and are undoubtedly related to the forc causing and modifying the flow (such as wind stress and tides); these w not be examined in detail here. However, the good coherence between drogu at the same depths (Figs. 3–8) suggests that the observed motion is a reliab indication of water movement.

Surface Motion. Fifteen drogues were placed at a depth of 10 m on t six stations. In all cases they moved rapidly out of the operational area a thus were followed for relatively short periods (5–23 hours). On all but t southernmost station, surface drift was toward the northwest (270°–353 with mean speeds of 11–29 cm/sec. This motion is consistent with the gen ally accepted picture of surface flow in the Peru Current.

On the southernmost station, average surface drift was toward 067° at speed of 19 cm/sec; during the first eight hours motion was toward the ea but as the drogues approached the coast they swung toward the northe and speeded up. Offshore the eastward motion (090° ± 4°) was at a speed 16–17 cm/sec. Five days later, at a position 35 km west of the drogue stati

Figure 6. Uncorrected drogue trajectories, St. D (13°51′S, 76°43′W); symbols as in Fig. 3.

surface current measurement by geomagnetic electrokinetograph[3] gave a locity of 13 cm/sec toward 097°, consistent with the observed motion of e surface drogues.

Subsurface Motion. In most cases subsurface movement was significantly fferent from that at the surface. At four stations, the deepest drogues moved

3 On the other stations such measurements were not possible because of the proximity of the magnetic uator.

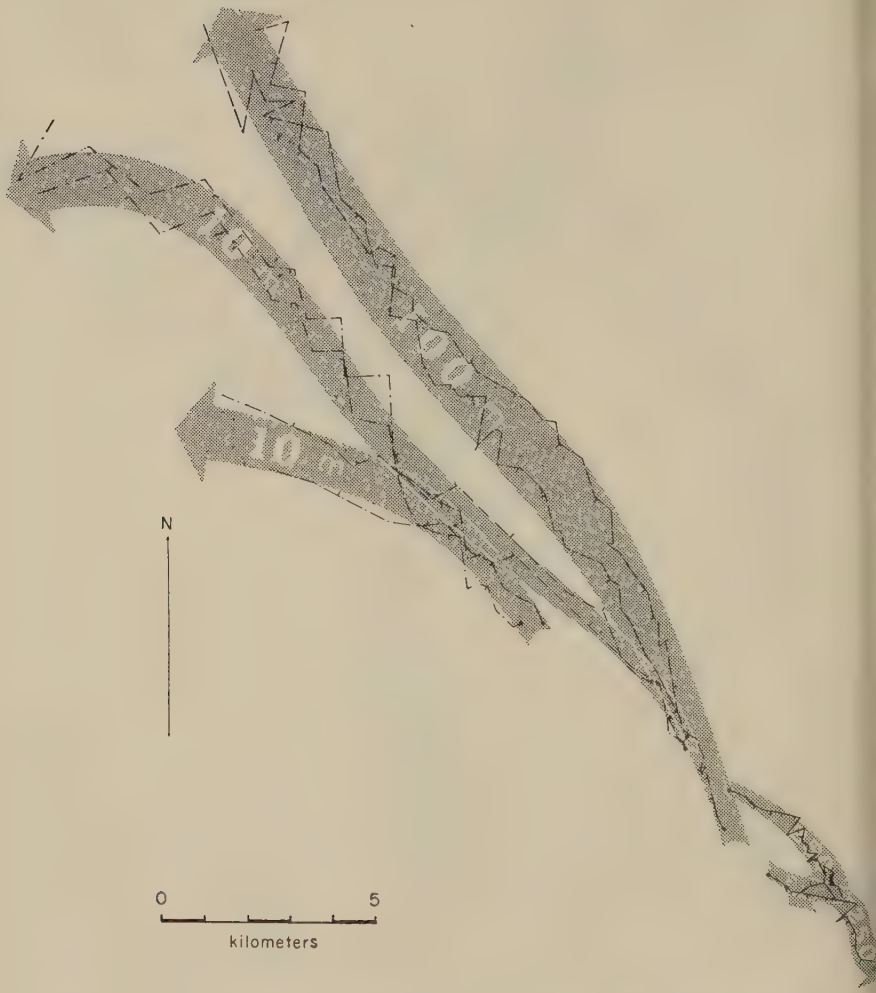


Figure 7. Uncorrected drogue trajectories, St. E (17°00'S, 72°15'W); symbols as in Fig. 3.

southward (133°–211°) at speeds of 5–9 cm/sec; on a fifth station (13°51'S corrected motion was also southward but at almost negligible speed (1 cm/sec and on the sixth (11°58'S), motion was toward the northwest. On thre stations, the drogues moved southward (160°–225°) at 4–8 cm/sec at inte mediate depths and faster at greater depths, suggesting that the princip motion lies somewhat deeper than 100–150 m.

Thus on all but one station there was some indication of a subsurface cu rent flowing southward along the coast in a direction more or less opposi to that of the surface flow. The anomalous station (11°58'S), with motio

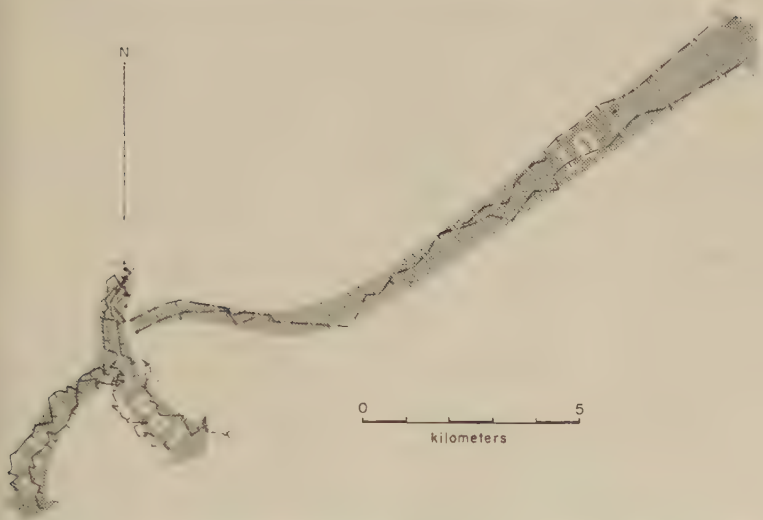


Figure 8. Uncorrected drogue trajectories, St. F (23°31'S, 70°53'W); symbols as in Fig. 3.

all levels toward the northwest, was the shallowest one occupied, and was ated off a wider continental shelf than were the other stations.

The direction of subsurface motion is closely related to the direction of e 100 fm contour (Fig. 9). This relationship is particularly striking on the tions at 17° and 23°30'S, between which there occurs a major change in entation of the edge of the continental shelf.

Evidence from Distribution of Properties. A southward current below the face along the west coast of South America has been discussed by Gunther 936), whose principal evidence for this circulation is a meridional profile salinity about 100 miles offshore, extending from 2°–35°S (his fig. 42). he high salinity tongue reaching southward from about 15°S is con- ered indicative of a coastal return current which deepens as it flows south- rd – from about 100 m at 15° to 300 m at 35°S. Other character- cs of this water are said to be relatively high temperature and low oxy- n content.

A profile analogous to that of Gunther has been constructed (Fig. 10), as south as 24° from STEP-I data, and to 41°S from Chiper and Ob uise 3 data (manuscript data from U.S. Hydrographic Office and World ta Center A, respectively). Evident here also are the high salinity tongue ching south from 15° to at least 41°S between low salinity waters of southern gin and a downstream decrease from greater than 34.8‰ at 15°S to less n about 34.5‰ at 41°S. At the same time thermosteric anomaly at the e of the tongue decreases, from 160 to less than 140 cl/T (centiliters per

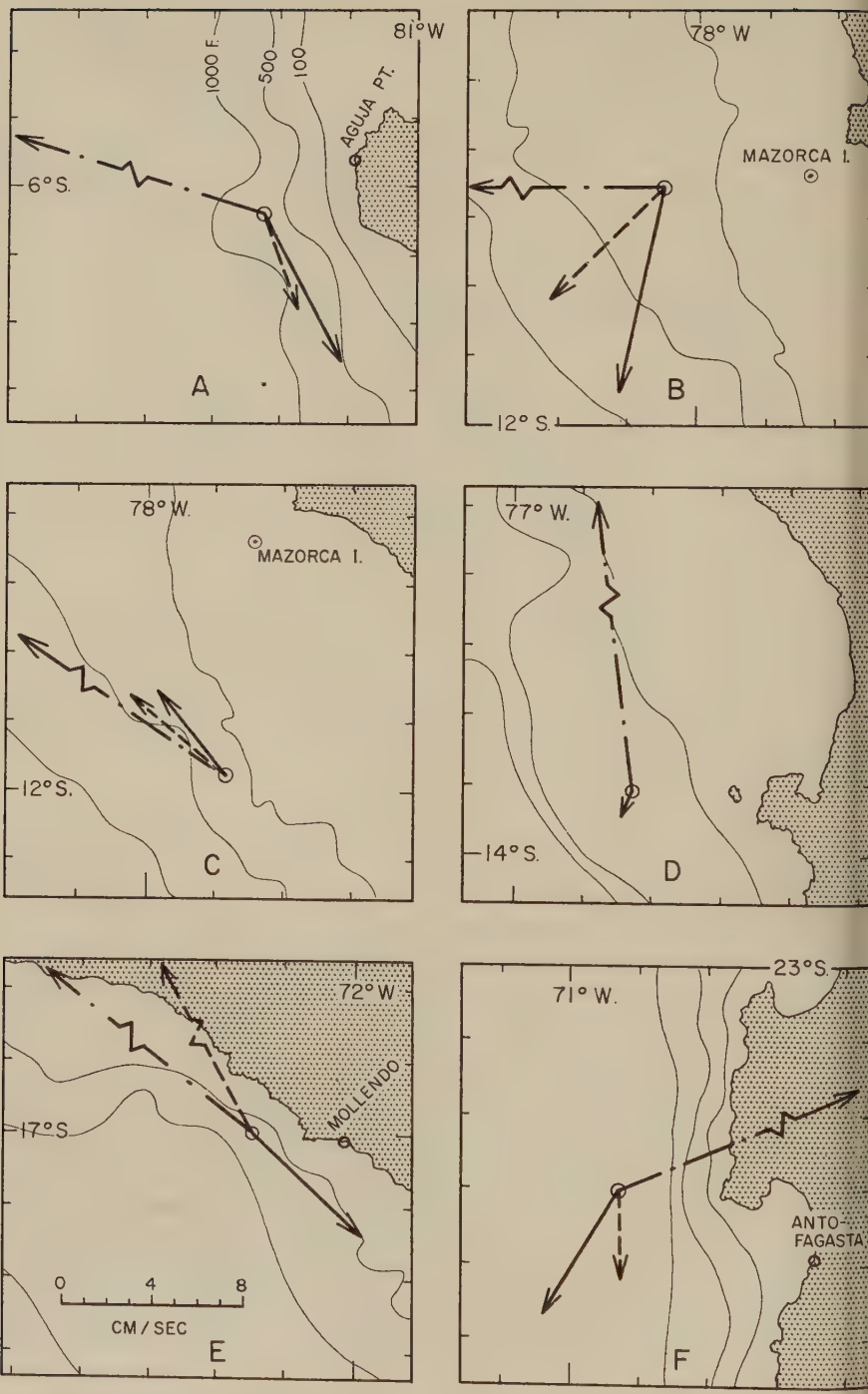


Figure 9. Corrected mean current vectors and bottom topography. Surface drogue vectors (not to scale) indicated by dash-dot lines, intermediate vectors by dashed lines, and deep vectors by solid lines.

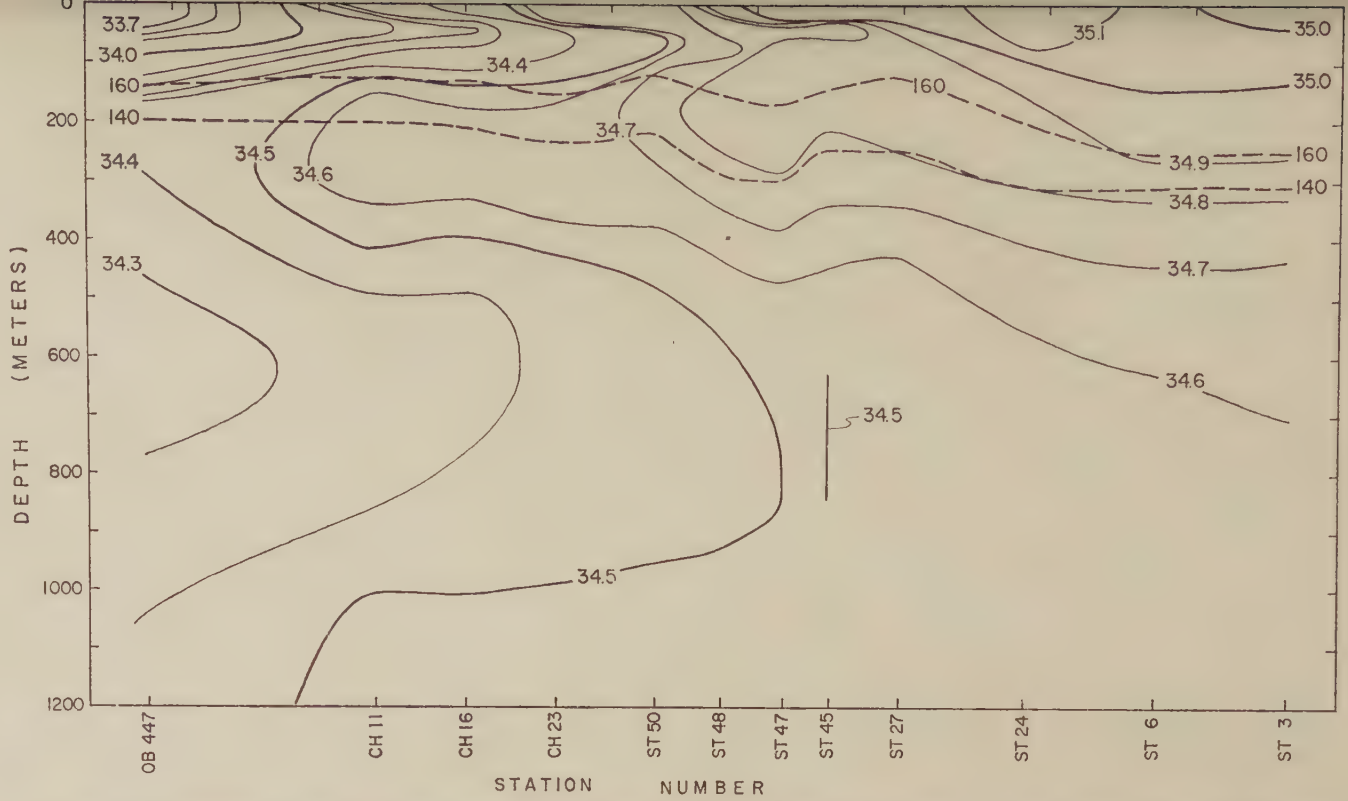


Figure 10. Distribution of salinity (‰) on a profile parallel to the west coast of South America about 100 miles offshore. Stations from STEP-I, CHIPER, and OB' Cruises (see text). Isanosteres of 140 and 160 cl/T shown by dashed lines.

ton), suggesting that attenuation of the tongue is due to mixing with wa of lower temperature.

A profile of dissolved oxygen from the same data sources (Fig. 11) sho clearly the low oxygen content of the southward-moving water in contr to the much higher values in the underlying water. Off the Peruvian co subsurface water contains about 0.1 ml/L of dissolved oxygen; as the wa moves southward, mixing with deeper water causes the oxygen content the minimum layer to increase to about 1.7 ml/L at 41°S. Highest values salinity and lowest values of dissolved oxygen are observed next to the co (Figs. 12 and 13) with attenuation of the maximum and minimum offsho Width of the high-salinity, low-oxygen tongue decreases downstream. At t southern end of the profile the undercurrent is also evidenced by phospha and silicate maxima (see Ob' St. 447 data).

Evidence of an undercurrent can also be seen in the profiles of geostrop velocity based on STEP-I data. In making the computations, thermoste anomaly was used rather than specific volume anomaly, this approximati being considered adequate for the purposes of this paper (Montgomery a Wooster, 1954). A reference level of 1000 db was used; although moti on this surface is not known, it is probably small relative to that of the ov lying water.[4] The use of a conductivity bridge to determine salinity redu the effect of measurement errors on geopotential anomaly to less than 0. dyn m (Wooster and Taft, 1958), and reporting relative geostrophic spe to the nearest few cm/sec may be justified.

Geostrophic speeds perpendicular to the five profiles extending offshore a shown in Figs. 14–18. Speeds less than 2 cm/sec have been considered neg gible. Some southward motion is apparent in each of the profiles; it predo inates in the northernmost profile and becomes progressively less intense the south. Highest southward speeds, shown in the coastal portions of t profiles, usually appear at depths of 100–200 m. In Profiles II and III, t principal southward flow seems to separate from the coast, although so southward motion is still apparent between coastal station pairs as far sou as Profile IV. On Profiles IV and V, southward geostrophic flow is ve much reduced. Speeds decrease from 20 cm/sec in the northernmost pro to about 2 cm/sec on Profile IV. Southward transport decreases from abo 21×10^6 on Profile I to about 3×10^6 m^3/sec on Profile III.

Discussion. Results from the parachute drogue measurements and fr analyses of the distribution of properties and the geostrophic computati demonstrate clearly the presence of a southward undercurrent along the co of Peru and Chile. Characteristics of the undercurrent can be summari as follows:

4 In one instance (see Appendix) the motion of a 1000 m reference drogue was estimated t 1 cm/sec or less.

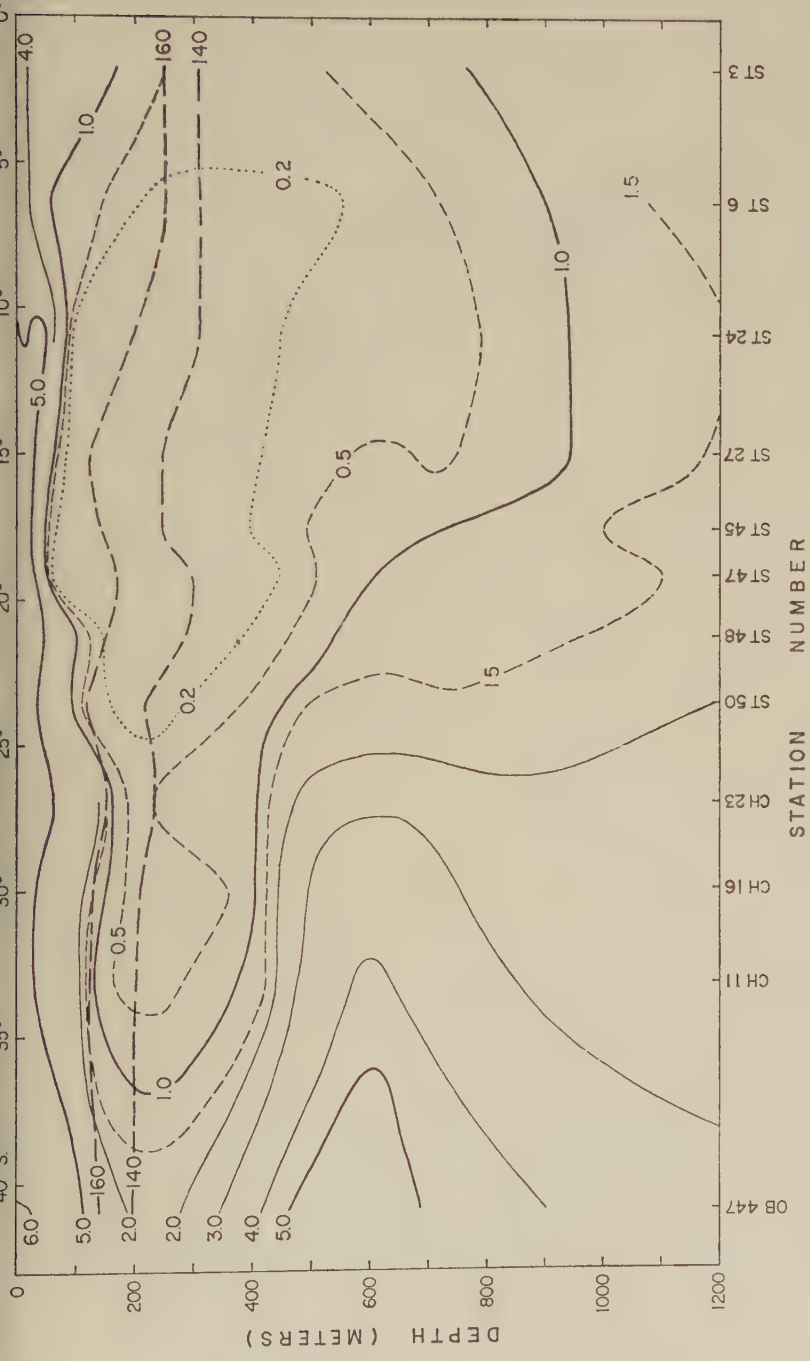


Figure 11. Distribution of dissolved oxygen (ml/L) on same profile as Fig. 10.

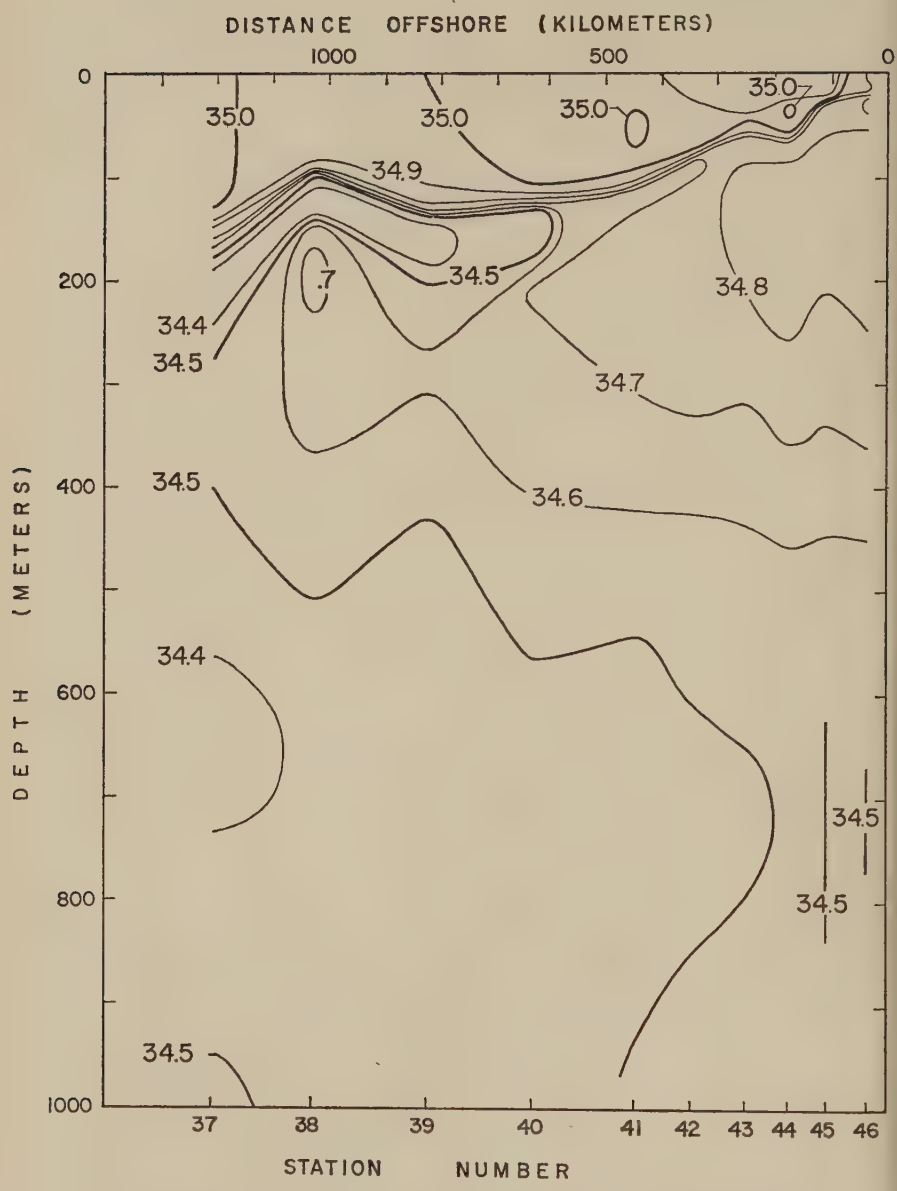


Figure 12. Distribution of salinity (‰) on Profile IV; see Fig. 2 for location.

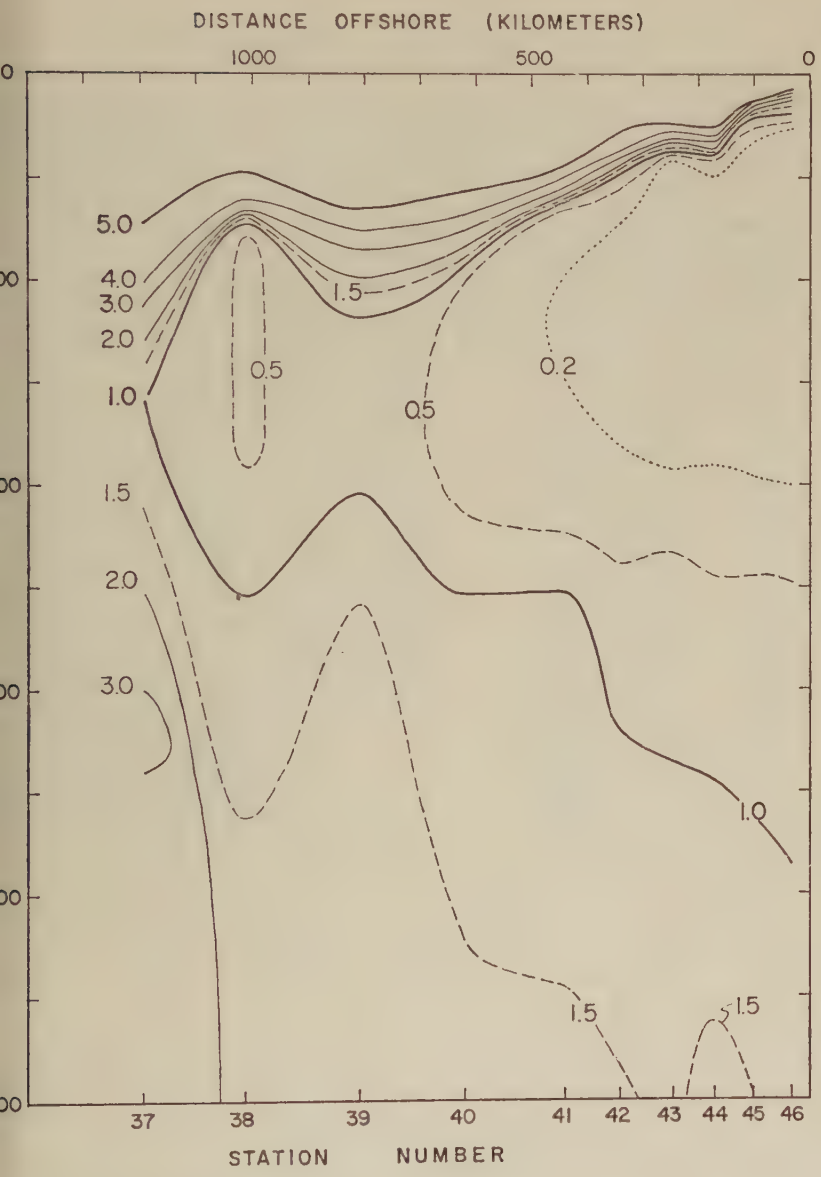


Figure 13. Distribution of dissolved oxygen (ml/L) on Profile IV; see Fig. 2 for location.

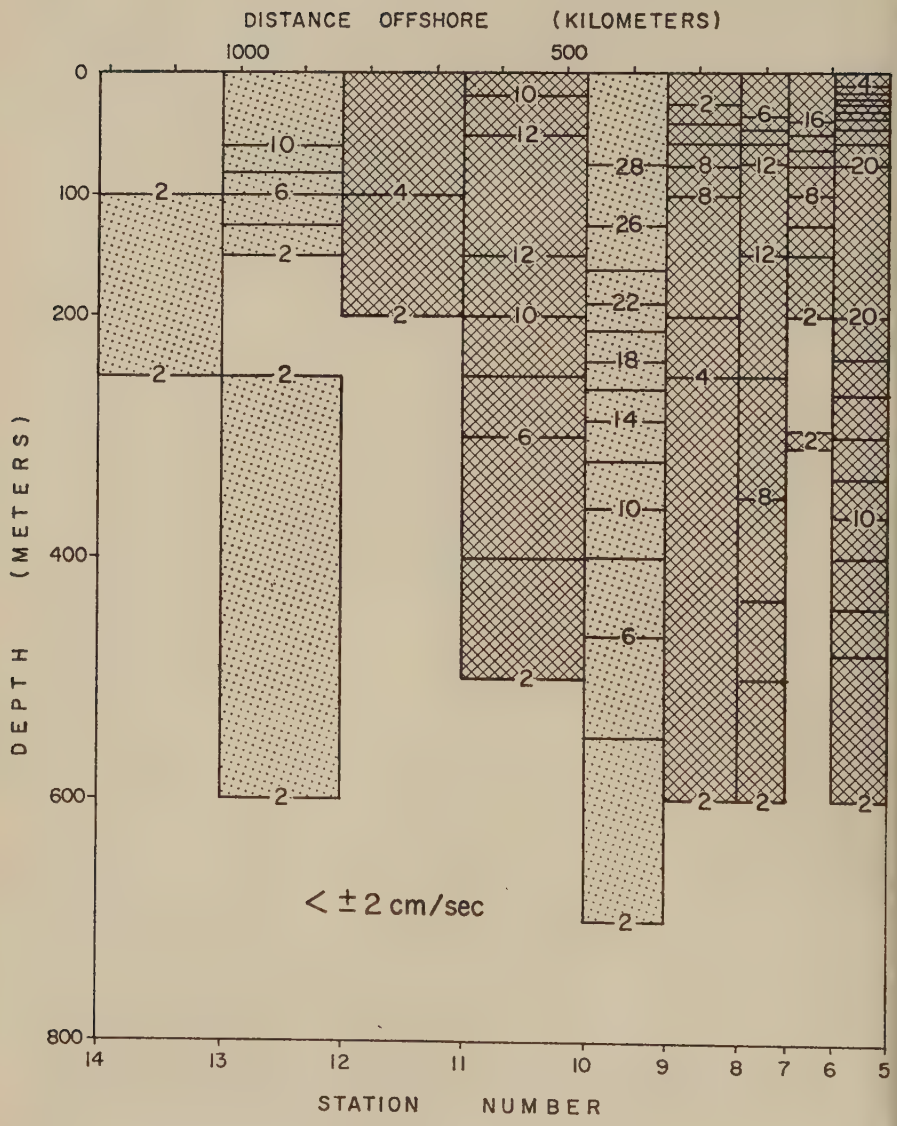
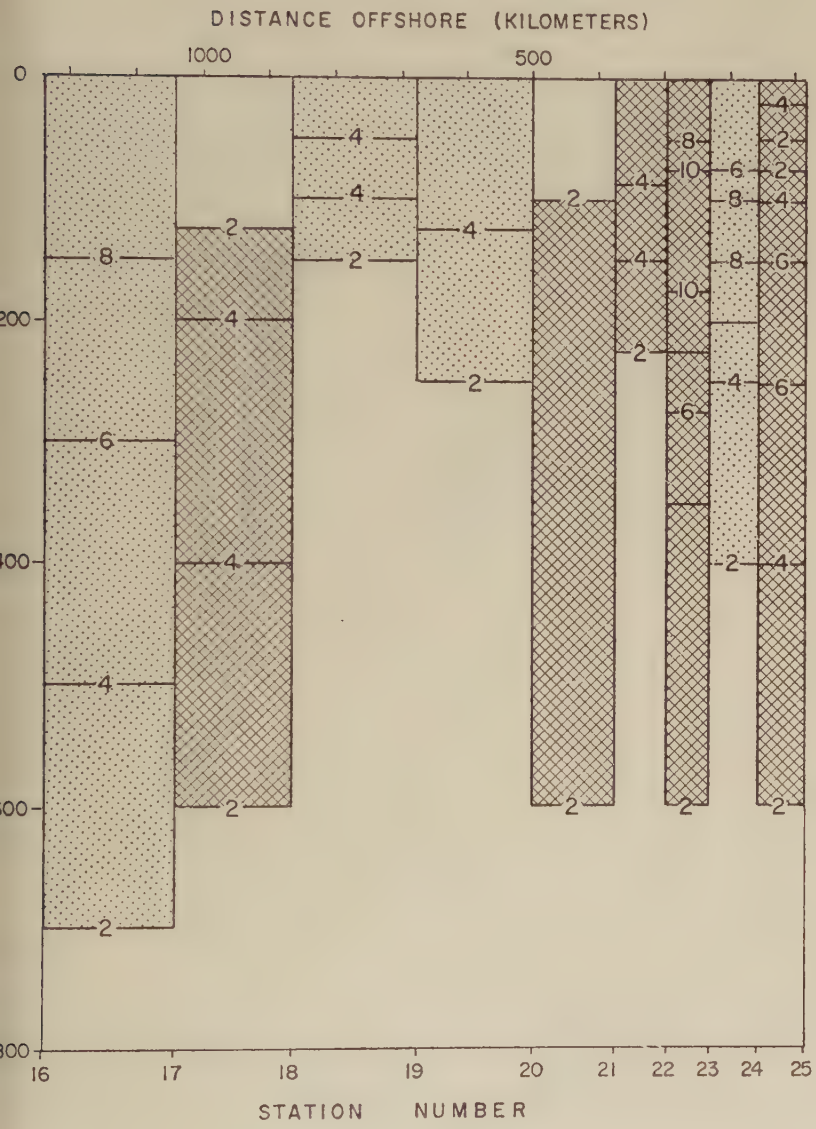


Figure 14. Geostrophic speed (cm/sec) perpendicular to Profile I; see Fig. 2 for location. Darke shading represents southward motion.



gure 15. Geostrophic speed (cm/sec) perpendicular to Profile II; see Fig. 2 for location. Darker shading represents southward motion.

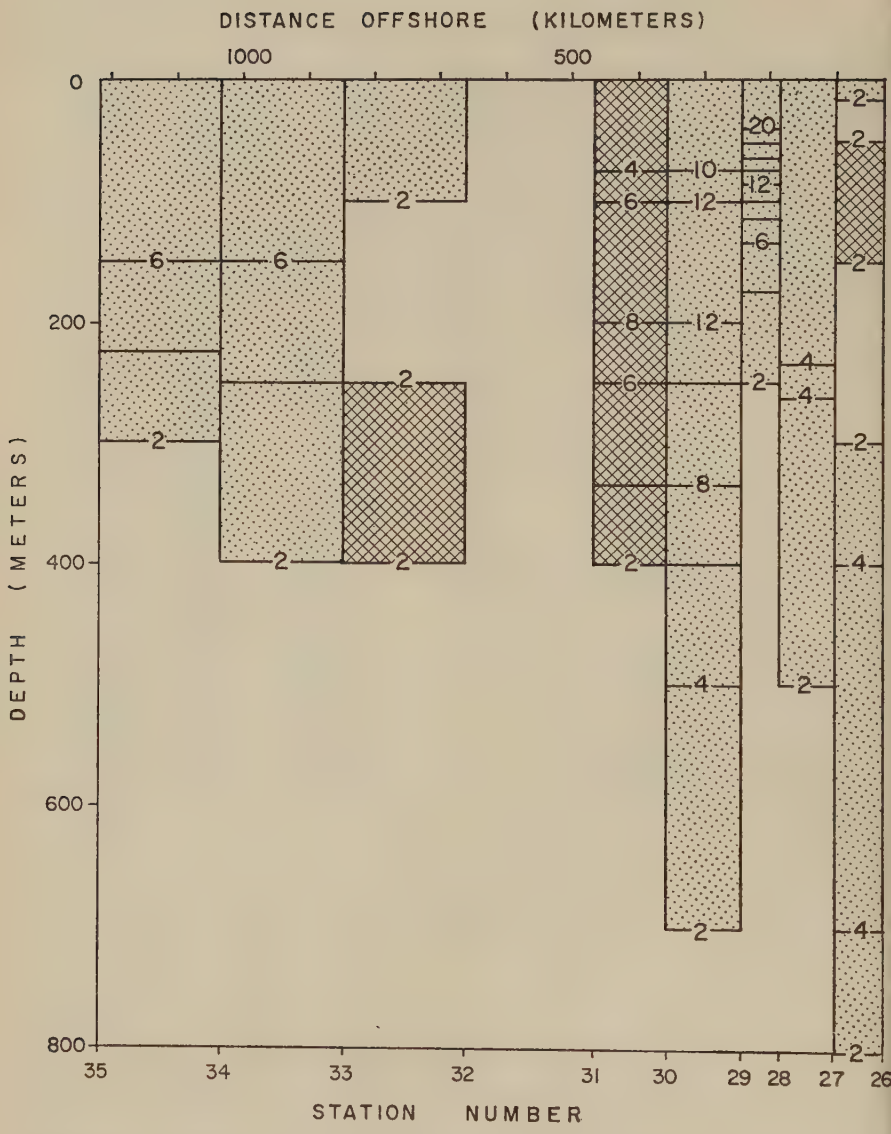


Figure 16. Geostrophic speed (cm/sec) perpendicular to Profile III; see Fig. 2 for location. Darke shading represents southward motion.

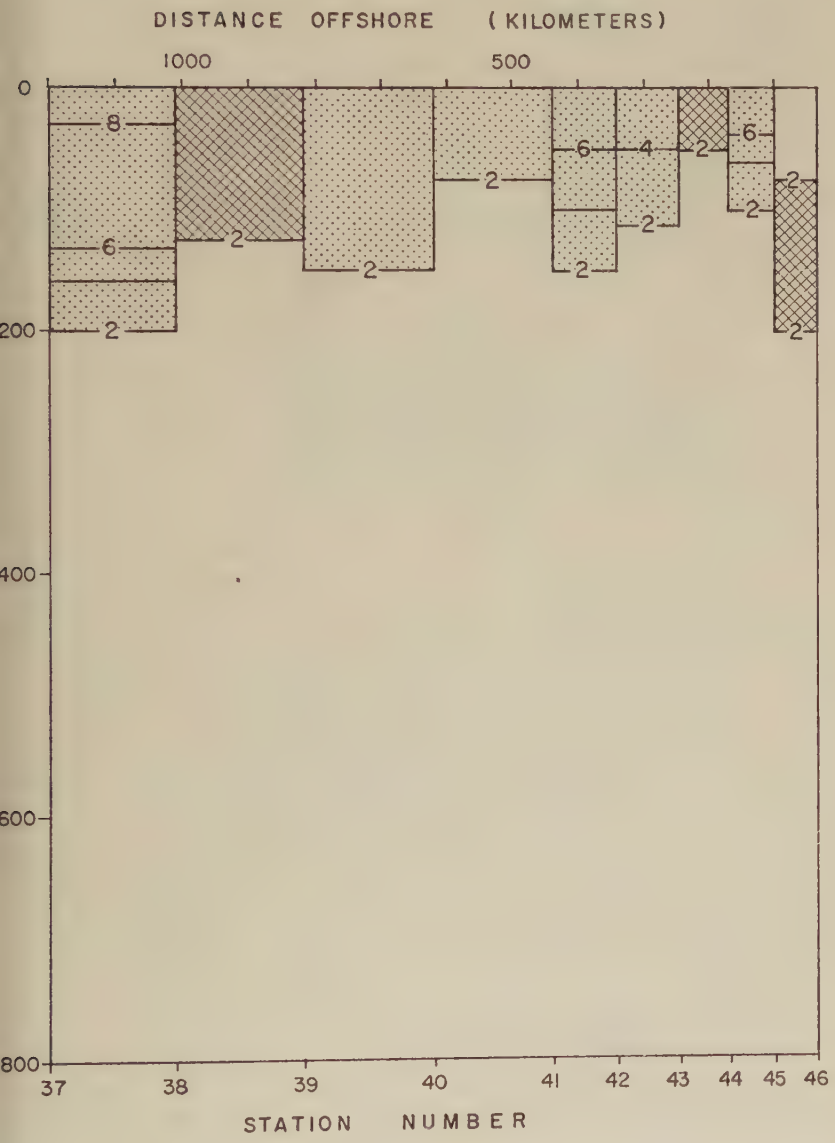


Figure 17. Geostrophic speed (cm/sec) perpendicular to Profile IV; see Fig. 2 for location. Darker shading represents southward motion.

DISTANCE OFFSHORE (KILOMETERS)
0
500
1000
1500
2000
2500
STATION NUMBER
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
DEPTH (METERS)
0
200
400
600
800
< ± 2 cm/sec.

1. Flow originates off northern Peru, in the region of the boundary of the ru Current or farther north, and extends at least as far south as 41°S at ɔths of several hundred meters.
2. Speed and transport of the geostrophic flow decrease from north to south. ıe geostrophic and measured speeds (4–10 cm/sec) are, in general, compatible cept on the northernmost profile where the geostrophic speed (20 cm/sec) significantly higher.
3. At its coastal edge, the flow closely parallels the edge of the continental lf. On the northern profiles, principal flow appears to separate from the coast.
4. South of about 15°S, waters of the undercurrent are characterized by atively high salinity and temperature and low oxygen content. Off southern ile the undercurrent is also evidenced by phosphate and silicate maxima.

The strong southward flow observed on the northernmost geostrophic ɔfile suggests that there is a supply of warm, high-saline water from the uatorial region north of the Peru Current regime. Between there and 15°S, undercurrent is presumably associated with the boundary processes of up-lling and related enhanced vertical mixing. The significant decrease in nsport of the geostrophic current from Profiles I to III suggests that much the water may be brought to the surface in the upwelling process in addition that lost by zonal transport.

The surface geostrophic flow to the south on the inshore end of Profile I es not appear to be compatible with the motion of the surface drogue (Fig. 9) with the presence of upwelling. The high surface phosphate content (2.05 μg L) as well as the vertical distribution of density and other properties there ggest that vigorous upwelling may have occurred just prior to our observa-ns. There is little evidence of upwelling on Profile II, but the inshore end of ɔfile III shows clearly all the usually accepted indications of the process.

It should also be noted that the flow measured with the parachute drogues l the flow estimated from the distribution of mass, although related, are the same. In general, the drogue measurements were close to shore in atively shallow water and pertained to rather specific time intervals and ers of water. The geostrophic currents, computed from the station pairs ng farther offshore in deeper water, involve a certain averaging of con-ons over distances of 40 miles or more. In the immediate vicinity of the ındary, the geostrophic approximation may not be applicable because of its glect of frictional forces, accelerations and vertical velocity.

South of 15°S, on Profiles IV and V, there are no indications of upwelling. is is in agreement with the findings of Wooster and Reid (in press) who wed that except in winter, there is little or no Ekman transport offshore ween 15° and 30°S. In this region an overflow of low-salinity water from coastal current off Chile and the presence at depth of cold, low-salinity ter make the undercurrent apparent as a layer of high salinity.

Despite the relatively small volume of southward flow, particularly soutl of 15°S, waters with the characteristics of the undercurrent are widespread Nothing is known concerning seasonal variations in the strength of southwar flow, and it is conceivable that this may be stronger at some other time o year than October. A few of the drogue measurements suggest that stronge flow is present than is indicated by the geostrophic data. It is also possibl that even a slow, and possibly intermittent, subsurface flow may be capabl of developing the observed distributions of properties. Certainly the decreas in volume of southward flowing water mentioned above suggests a continua loss through vertical and horizontal transport.

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APPENDIX

ccuracy of Parachute Drogue Measurements

In estimating the absolute motion of water at a certain depth by means parachute drogues, one is faced with (1) errors in determining the uncor-cted motion of a drogue relative to the reference point, and (2) errors in sitioning of the reference point itself. In addition there may be systematic ors in the correction of the apparent relative motion for the drag forces ing on the drogue system. We have considered as unimportant for the esent problem the uncertainty of the magnitude of these drag forces and have cepted the empirical values of Brown (in press) without modification.

In determining the motion of a drogue relative to the reference point, the mary errors are in range and bearing as measured by radar. The inherent curacy of the equipment itself is high, but random errors arise from operator erpretation of the radar targets. Such errors depend on operator experience, state, condition of the drogue's radar reflector, tuning of the radar set, . A measure of these errors can be obtained from analysis of the duplicate dings of range and bearing of the reference point, customarily taken every f hour or so while on a drogue station.

The within-replicate variance, σ^2, for a large number of replicates is imated by the equation

$$\sigma^2 = \frac{\sum (x - y)^2}{2n},$$

ere x and y represent the replicate values and n the number of replicate s used in the analysis. The results of such analyses for each drogue station included within the total error presented in Table I.

These errors can be assumed to be independent of range. The total error in sitioning the drogue relative to the reference is the sum of the errors in sitioning the ship (S) relative to the reference (R), and the drogue (D) ative to the ship. The S–R relation is determined from the mean of duplicate asurements of range and bearing, the S–D relation by a single measurement; total error in the drogue position, E, is given by

$$E = 2\sqrt{\frac{\sigma^2}{2} + \sigma^2} = \sigma\sqrt{6}.$$

imates of total error are presented in Table I.

Errors in positioning the reference point depend on whether an island or eep parachute drogue was employed. In the former case, the island is not oint target and may appear to shift in position when viewed from different rings. The islands selected in this study were small and precipitous and a sufficient distance so that we feel such errors can safely be neglected.

When it was necessary to use deep (1000 m) parachute drogues as reference the radar reflector was essentially a point target, but the assumption of negligible motion at such depths may not be valid. On one station (6°S) a rough estimate of reference drogue motion was made by bringing the ship alongside the reference drogue on three occasions and by determining the ranges and bearings of four points on the shore. Although these shore points were not ideal for this purpose, the successive measurements showed an average southward movement of 1–2 cm/sec. No correction has been made for this apparent change in reference position, but such a correction would serve to increase slightly the estimated speed of the undercurrent.

In treating all of the drogue data it was assumed that all ranges and bearings of a given set were measured simultaneously. In practice, 5–6 minutes were required for the measurements, during which the ship may have drifted approximately 70 m (20 cm/sec for 6 min). Since the ships position relative to the reference was determined by averaging measurements at the beginning and end of the set, the drift error was of the order of 35 m, a small value relative to other errors.

The final positioning of the drogue depends on the coupling of the reference point-ship vector to the ship-drogue vector. Because of errors in range and bearing, the probable position of the ship or of the drogue will lie somewhere within an annular area, with the difference between the concentric circles representing the error in range, and the enclosed angle between the radii representing the error in bearing. The total error in the final drogue position will result from the coupling of these areas. The result is a function, not only of the size of errors in ranges and bearings, but of the size of the angle enclosed by the two bearings. The solving of such vector coupling is a complex operation and for simplification it was assumed that the position of the ship lay somewhere within a circular area representing both error in range and error in bearing. The coupling of two such circular areas is independent of the angle enclosed by the bearing. The larger of the two errors resulting from range and bearing determinations was taken as the radius of the circular area. Provided that the two errors differ by at least 15%, the situation at all stations, such an assumption will result in an overestimation of the error in final drogue position and is therefore considered justified.

The Measurement of Dehydrogenase Activity in Marine Organisms[1]

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Abstract. A method for the estimation of respiratory potential in marine biological material iscussed. The method, which depends upon photometric assay for a dehydrogenase en-ne by the reduction of tetrazolium salt, showed that activity measurements are positively, arly, related to oxygen consumption measurements. Respiratory potential may be asured immediately upon securing the material, or at a later time.

INTRODUCTION

Studies of rates of energy transfer and metabolic turn-over in marine pop-tions are dependent upon measurements of respiratory rates of samples of populations or their components. The standard procedure for assessing h metabolic activity has been the measurement of oxygen consumption by techniques of iodimetry (Winkler method), manometry, or by oxygen ctrode. Among them, the techniques involve variables and inconsistencies t render intercomparison difficult. The most important variable is biolog-: the crowding of organisms at high density in a bottle, sometimes for ended periods, is a very unnatural condition. Since oxygen consumption lf is a function of the chain of respiratory enzymatic reactions interposed ween oxidation of a substrate and the assimilation of oxygen, the unusual ditions present during the usual measurement of oxygen consumption may avoided by determining the activity of one of the interposed enzyme systems ier *in vitro* or *in vivo*.

Contribution No. 1209 from the Woods Hole Oceanographic Institution. This study was aided by ts from the National Science Foundation (NSFG13272 and G8339).
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Woods Hole Oceanographic Institution, Summer Student Fellow, 1960.

Some enzyme systems, *e. g.* the cytochromes, do not lend themselves to th "quick and dirty" methods that must by employed on shipboard, if not in th laboratory itself, because of their lability. However, a variety of biochemic techniques for the determination of dehydrogenase activity have appeared recent years, and these are admirably suited for use with plankton organism Initially, the rate of reduction of redox dyes such as methylene blue was use although there was no satisfactory evidence that a single component of coupled enzyme system was being determined. Tetrazolium salts have foun favor in recent years for determining dehydrogenase activity in histochemic preparations because the reaction could proceed aerobically and the resultin colored products could be determined with great sensitivity, although th reaction is not necessarily specific for a particular substrate (Novikoff 195 Cooperstein *et al.* 1950).

Aleem (1955) attempted to determine the standing crop of phytoplankto by using a tetrazolium salt, assuming a constant respiration rate per unit weig of organic matter.

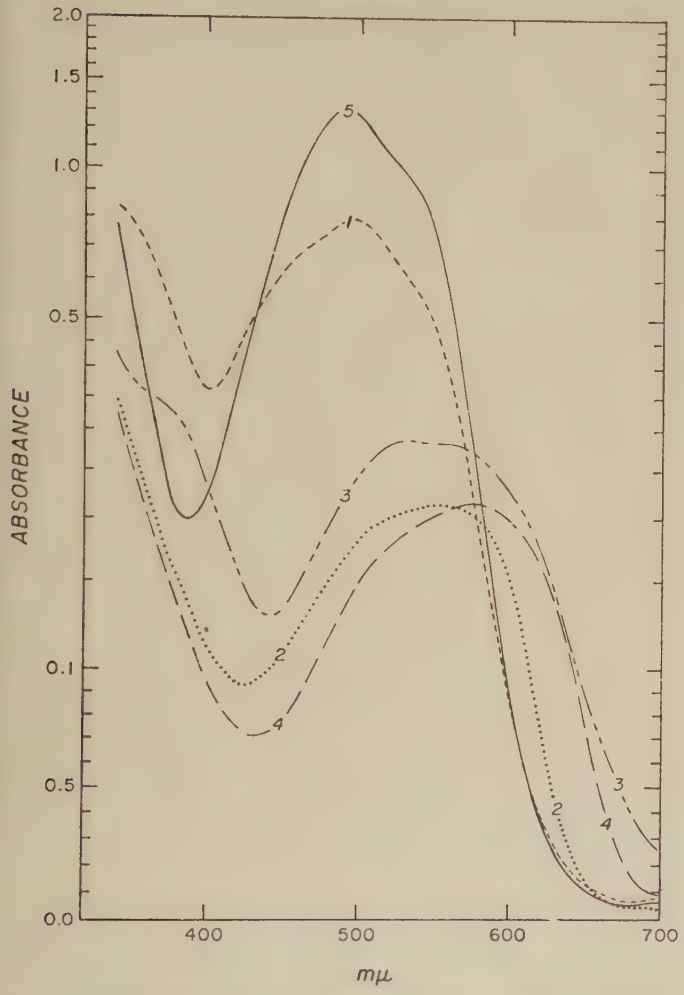
The tetrazolium salts are organic compounds which form stable colore compounds (formazans) upon reduction. This may be accomplished wit various organic compounds such as ascorbic acid, or substrates or DPNH the presence of flavine-containing enzyme. In aerobic metabolism, energy obtained from a stepwise transfer of electrons from complex organic compoun to oxygen, the process of oxidative phosphorylation. Dehydrogenases oxidiz or remove hydrogen atoms in a number of these steps. The tetrazoliums ha the convenient attribute of intercepting hydrogen atoms, thereby becomin themselves reduced instead of the associated cytochromes. The reductio products, formazans, occur as water-insoluble precipitates in tissues, usual close to the sites of actual dehydrogenase activity. For quantitative purpose the colored formazan may be extracted in an organic solvent and the concen tration determined photometrically.

The objective of the study reported here was to develop a quantitati procedure for measuring the dehydrogenase enzyme activity in living tissu of phyto- and zoo-plankton at sea, as an indicator of the respiratory potenti of the populations.

METHODS

Reagents. A variety of tetrazolium salts is commercially available and ne ones appear at frequent intervals. Among those tested were TTC (Triphen tetrazolium chloride), Tetrazolium blue, Neo-tetrazolium chloride, Nit blue tetrazolium and INT (2p Iodophenyl-5-phenyltetrazolium chloride) Aqueous solutions of the salts were reduced with ascorbic acid, note bein taken of the length of time required to produce a dense colored product. Th

4 All obtained from the Nutritional Biochemicals Corporation, Cleveland, Ohio.



ıre 1. Absorption spectra of five formazans. Tetrazolium salts were reduced with ascorbic acid under aerobic conditions and the resulting formazan dissolved in TCE-acetone. INT (1), Neo-T (2), Nitro-BT (3), Tetrazolium Blue (4), TTC (5).

ılting formazans were extracted with a tetrachloroethylene-acetone mix-e, and absorption curves were plotted with a Carey recording spectrophoto-ter (Fig. 1). INT and Neo-tetrazolium reduced immediately. Comparison INT reduction under aerobic and anaerobic conditions showed no differ-e in amount of formazan formation. Nitro blue tetrazolium began reducing nediately but took about ten minutes to achieve maximum color. Blue azolium and TTC showed no color after one hour; however, upon raising

the pH, a formazan was produced very slowly by Blue tetrazolium, but n by TTC; in approximately 48 hours TTC also became reduced. Under ana robic conditions it seems likely that these latter tetrazoliums would ha reacted more quickly. INT was chosen for experimental purposes becau it competed well with oxygen for liberated electrons, making anaerobia unnecessary, because it was reduced most rapidly of those tested, and b cause it gave the greatest sensitivity. INT formazan has a rather narro peak of light absorption at 490 mμ and would be suitable for use with spectrophotometer.

An INT solution was prepared by dissolving 100 mg of the substance 50 ml of distilled water. A few drops of 3N sodium carbonate solution we added to bring the pH to approximately 7.5, thereby increasing the solubili of the salt. After filtering, the resulting clear, yellow-brown solution w kept under refrigeration. New solutions were prepared weekly, but if ke at 5°C they were stable for several months.

Sodium succinate was prepared weekly as a 0.4M stock solution in distill water with a few drops of 3N sodium carbonate solution added, and refrige ated. Other substrates were prepared in a similar manner.

Phosphate buffer of pH 7.7 (0.2M) was prepared from KH_2PO_4 a NaOH solutions.

Biological Materials. Both intact and homogenized organisms were use Intact organisms were wet-weighed and used immediately. Homogenates we prepared with a ground glass tissue grinder at concentrations of 25 mg of w tissue per ml of homogenate, in distilled water. Grinding was performed wi the apparatus immersed in ice. The homogenate was centrifuged for a fe minutes to provide a supernatant fluid devoid of visible particles; usually o ml of this fluid was used in a reaction.

Procedure. One ml of homogenate or approximately 25 mg of intact orga isms was added to a reaction mixture consisting of 2 ml phosphate buffe 1 ml 0.4M sodium succinate, and 1 ml of 0.2% INT solution in screw capped 15 ml test tubes; reaction mixture for one day was made up in the proportions in advance. No attempt was made to estimate the keeping pro erties of the reaction mixture beyond 24 hours. (Nachlas *et al.* [1960] fou that their combination of reagents would keep for two weeks at 5°C. A −18°C the reaction mixture remained unchanged at the end of three month The tubes were placed at once in a covered constant-temperature bath for carefully timed period.

The formazan produced is insoluble in water but is soluble in organ solvents such as isoamyl alcohol, chloroform, acetone and tetrachloroethylen A suitable solvent would be immiscible with water, would not form unbrea able or strong emulsions with water, especially in the presence of much di

ved organic material, and should have a relatively low vapor pressure. A 1.5 mixture of tetrachloroethylene and acetone satisfied these requirements. rmazan formation may be stopped either by addition of HCl to lower pH to 6 or simply by the addition of solvent. The usual procedure s to remove tubes from the incubator to an ice bath prior to addition solvent.

Eight mls. of solvent were added to each tube and permitted to stand for ious lengths of time with shaking. After brief centrifugation, solvent was etted from the tube into small screw-capped vials through filter paper. here appears to be no limit to how long the extracted formazan may be ot. Evaporation of solvent must be prevented, therefore discs of poly- ylene sheeting were used as gaskets in both the reaction tubes and in vials. Strong light will bleach formazan temporarily. Rather than work subdued light, solutions of formazan were kept in a dark box or under ck cloths.

Measurements of optical density of the extracted formazan were made at 0 mμ on a Beckman model B spectrophotometer against a reagent blank nsisting of all reagents and homogenate except INT.

Measurements of oxygen uptake were made in aspirator bottles of from 0–500 ml capacity. The experimental animals were acclimated for 15–30 nutes in the dark, with water flowing through the bottle before the exper- ent began. The Winkler method was used in oxygen determinations.

RESULTS

The optimal conditions for various parameters in the reaction were in- tigated.

pH. The pH of the reaction mixture was varied from 6.8–8.0 (Fig. 2). ing homogenates of the marine minnow *Menidia menidia*, an optimum of was found, but it should be possible to achieve good results throughout a ge of 7.5–7.9.

Incubation Temperature. Homogenates of *M. menidia* were incubated with T at temperatures of 25–50°C, with and without pre-incubation of the nogenate before addition of the reaction mixture (Figs. 3, 4).

Homogenates kept at 0–5°C showed no activity. When incubated at temp- tures of 25, 30, 35 and 40°C, increased rate of reaction was observed with reasing temperature, except that the rate at 40°C was initially the same as t at 35°C and declined after 20 minutes incubation (Fig. 3). Homogenates re incubated at temperatures of 25–50°C for 20 minutes without addition reaction mixture, after which they were incubated for 30 minutes at 34°C.

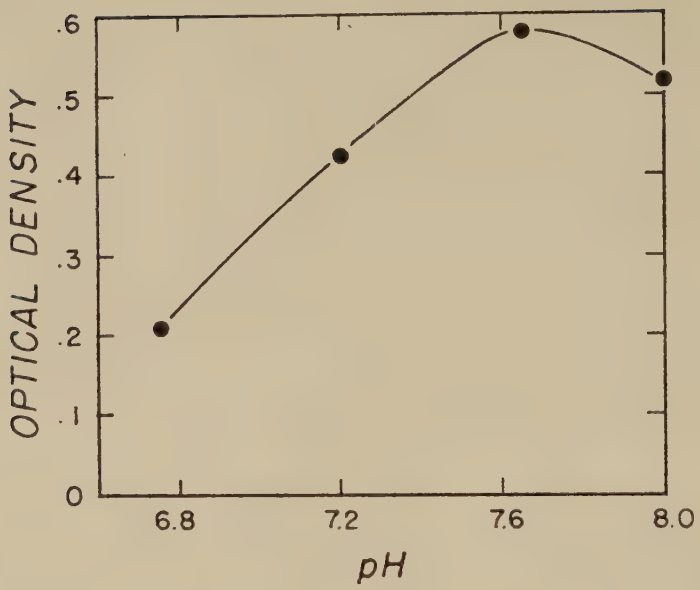


Figure 2. Reduction of INT as a function of pH; incubation 30 minutes at 35°C.

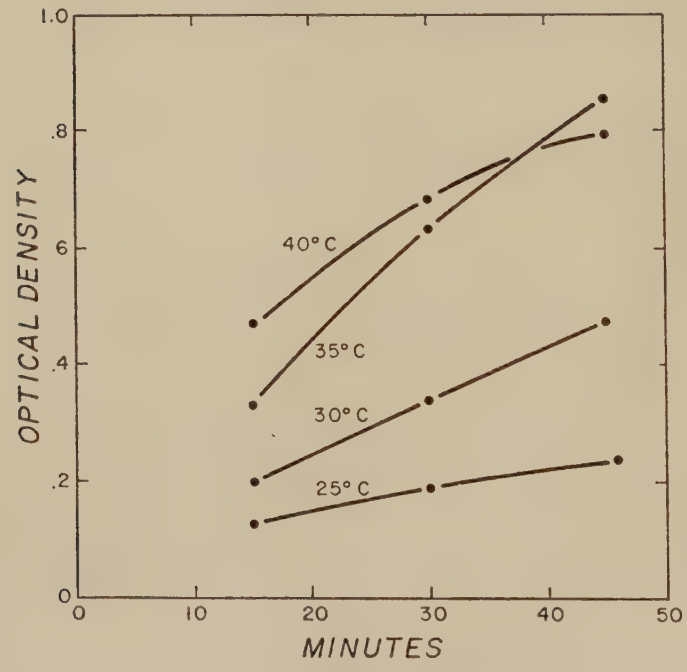
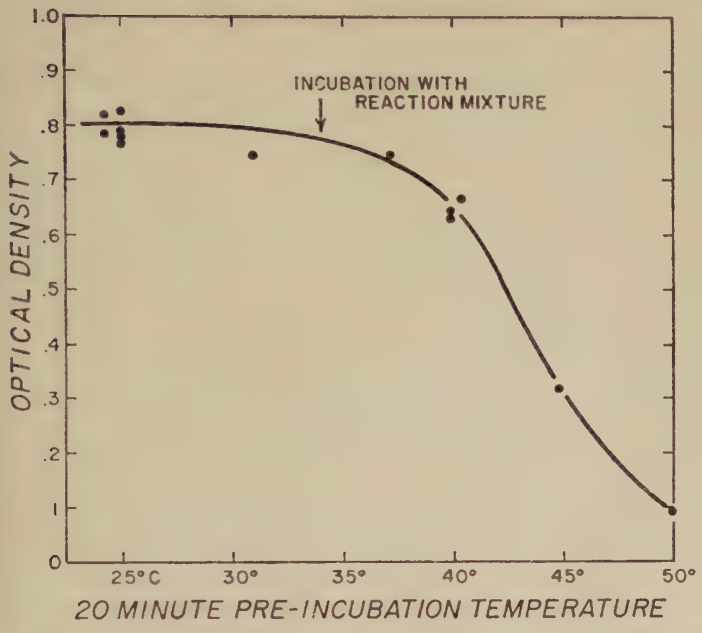


Figure 3. Reduction of INT as a function of time at various temperatures.



re 4. Effect of preincubation at various temperatures before addition of reaction mixture; reaction for 30 minutes at 35°C.

e amount of reduction decreased about 18% from 25–40°C. At higher peratures, activity was greatly reduced (Fig. 4). An incubation tempera- of 35°C was chosen for subsequent experiments and pre-incubation was ded inasmuch as no increase in activity resulted. This regime gave the imum rate of activity, linear with time, and gave no evidence of enzyme tivation.

Reagent Concentration. It was desirable that the measured enzyme activity ndependent of INT concentration. The concentration had to lie within limits of decreasing the reaction rate by its scarcity or by inhibiting the tion with an excess. The rate of reaction was measured with three stock centrations of INT: 0.08, 0.04, 0.01% at a temperature of 35°C and homogenate concentration of 0.025 g/ml (Fig. 5). An INT concentration .04% gave a reduction rate which was essentially linear for the first 30 utes and gave the highest rate among the three concentrations. A con- ration of 0.08% is still a very low one. Its obvious inhibiting action r 20 minutes may indicate that INT can so successfully compete for trons that the intermediary metabolism dependent upon dehydrogenation starved".

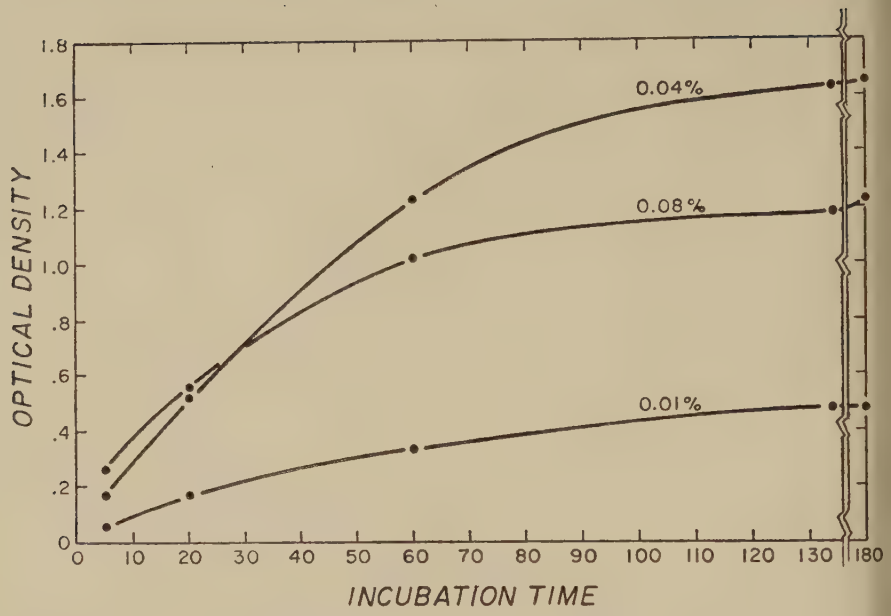


Figure 5. Effect of INT concentration and time on development of formazan, at 35°C.

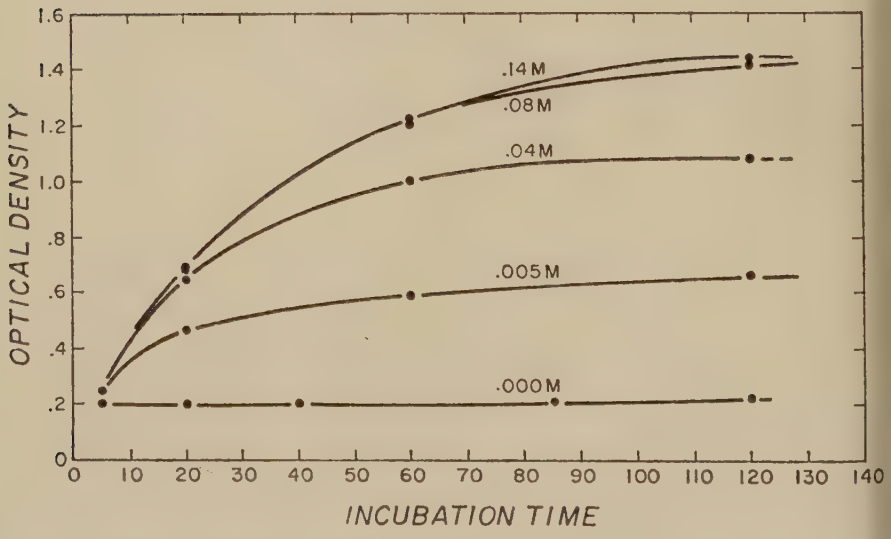
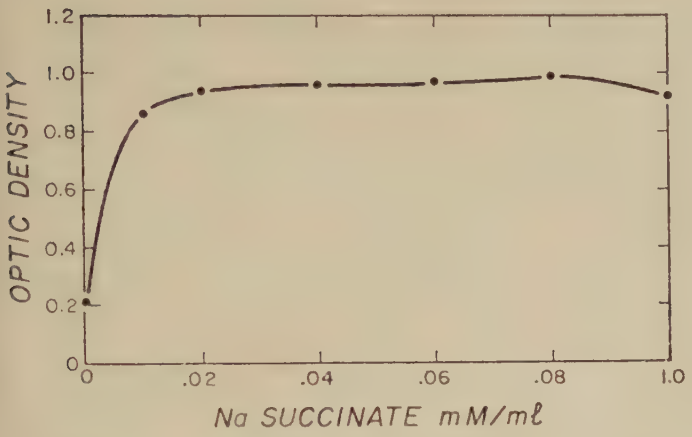


Figure 6. Effect of succinate concentration and time on development of formazan, using standard r
action mixture (see text).



ure 7. Effect of succinate concentration on formazan development, using standard reaction mixture.

Enzyme activity is proportional to substrate concentration up to some aximum and then becomes independent of further increase. It is particularly sirable to operate on a portion of the curve where the reduction rate is in-pendent of substrate concentration. Four concentrations of sodium succinate ere investigated (Fig. 6). The reaction rate increased with higher succinate ncentrations up to 0.08M. Doubling this concentration had no significant fect upon the reaction rates.

A curve of initial velocity versus substrate concentration (Fig. 7) indicated at the maximum velocity was reached at a substrate concentration of 0.02M r a 20 minute incubation, other conditions being fixed. The standard succinate ncentration was chosen to be 0.08M, for at this concentration the solution as substrate-saturated and no substrate inhibition was found below 1.0M ccinate.

Incubation Time. The initial velocity of the reaction may be obtained from curve of activity or optical density versus time (Figs. 3, 5, 6, 9). The initial locity is obtained by extrapolating the curve to t = 0. For the standard reac-on conditions, all the curves indicate linearity between activity and time up at least 30 minutes. One interval on the linear portion may be taken to present the initial velocity. The 20-minute interval was used as it was in e linear range and yielded a convenient optical density.

Enzyme Concentration. Presuming that substrate and indicator are present unlimited quantities and that aerobic metabolism may be carried out, the duction of indicator should proceed indefinitely and at a rate proportional the concentration of enzyme. However, in *in vitro* experiments, substrate

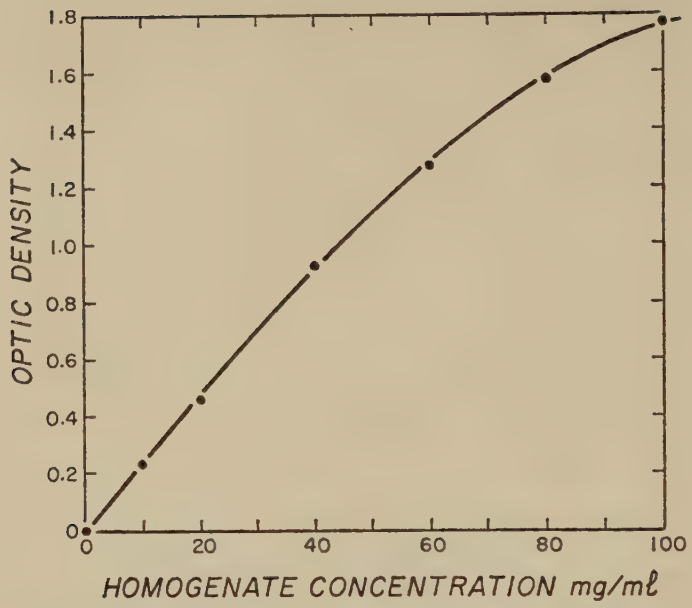
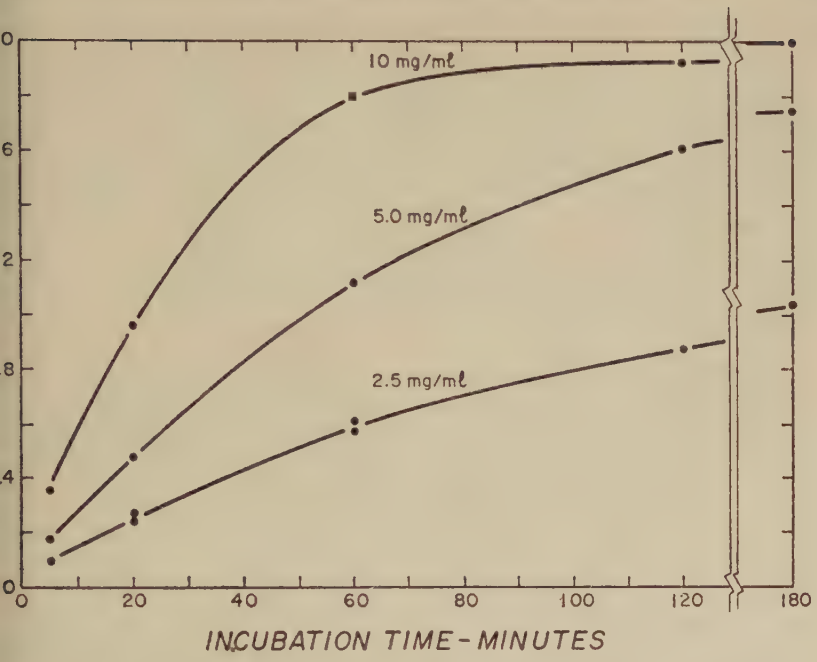


Figure 8. Effect of homogenate concentration on formazan development using standard reaction mixture.

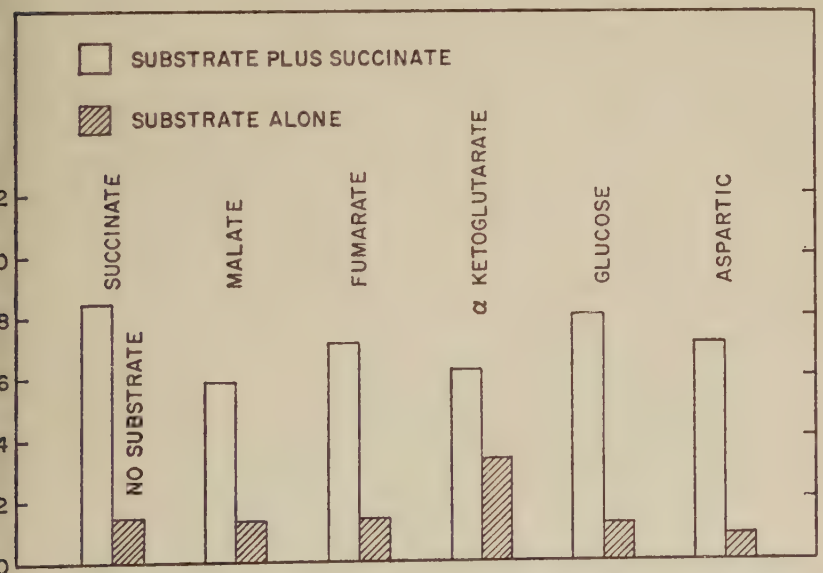
and indicator are both ultimately limiting and the rate decreases. For a definite amount of formazan production to be proportional to the enzyme concentration, the formazan must be measured while the rate of production is constant and is limited only by enzyme concentration.

By varying the concentration of *M. menidia* homogenate, a linear relationship was obtained between formazan production and enzyme concentration up to 60 mg wet weight/ml homogenate (Fig. 8). Several concentrations of *Menidia* homogenate were incubated for various lengths of time. The effect of reagent depletion was most noticeable at the highest concentration of 10 mg/ml homogenate, occurring after about an hour. The concentration of 2.5 mg/ml homogenate gave a slowly decreasing rate of reduction but no evidence of sudden depletion for at least three hours (Fig. 9).

Substrate Dependence. It has been reported that cofactors are required for the *in vitro* use of substrates other than succinate (Kivy-Rosenberg *et al.*, 1959). We have demonstrated that homogenized organisms require exogenous substrate for reduction of significant amounts of indicator. Without substrate, only very slight activity was observed (Figs. 7 and 10). Sodium succinate was used in these experiments. In addition, the effects of alpha keto glutarate, malate, fumarate, glucose, and aspartic acid were determined. The use of substrates other than succinate gave an amount of reduction equivalent to that



gure 9. Effect of homogenate concentration and time on formazan development, using standard reaction mixture.



gure 10. Effect of various substrates with and without added succinate on formazan development.

achieved with no substrate at all (Fig. 10). The combination of one of the other substrates with succinate gave values lower than the value obtained with succinate alone (with the exception of glucose-succinate). We may have some confidence that the method is specific for succinic dehydrogenase, since only this substrate with no cofactors has been used. Other endogenous substrates and their associated cofactors appear to be in too low supply to support significant reduction.

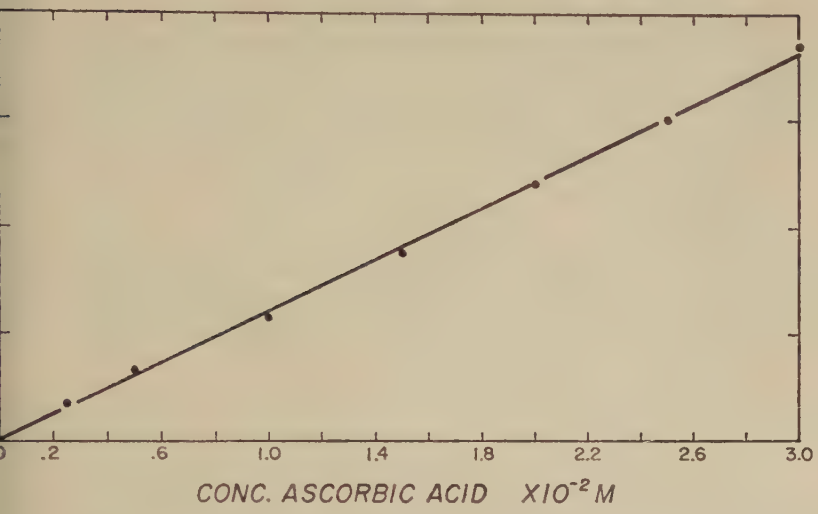
Reduction with intact organisms was less dependent upon an exogenous substrate source (succinate) than with homogenized organisms, but the addition of substrate accelerated the reaction by 10%.

Preparation of Biological Material. In order to adapt the technique to shipboard use, two additional problems were investigated: the feasibility of preservation of material for later analysis, and the desirability of homogenizing the sample either before or after reduction.

At 25°C, activity diminished by about 50% in 6 hours and was nil after 20 hours. A renewal of activity after 24 hours was observed as a result of bacterial growth. A high rate of activity may be preserved for 3 hours at 0°C but this will diminish by 50% in 48 hours.

Freezing intact and homogenized organisms with dry ice at –60°C to maintain high activity was unsuccessful. Intact specimens appeared to give consistently lower values than homogenates.

Calibration. In the experiments described thus far, it has been shown that a good correlation exists between the quantity of biomass used and the resulting formazan production. However, we have demonstrated that the reaction is dependent upon a single substrate-specific enzyme, succinic dehydrogenase. The quantities of biomass used have been equated with corresponding quantities of enzyme, but no direct estimate of the actual enzyme content was made. Except as has been done indirectly in these experiments, no such estimate is feasible. It is possible either to measure the actual proton-accepting ability of a given amount of tetrazolium or to determine the amount of formazan produced proportional to the availability of a given number of protons. The latter course has been followed. Aliquots of an ascorbic acid solution providing 0.1 meq. H^+/ml were added to the standard reaction mixture and incubated until color formation had ceased (Fig. 11). Ascorbic acid in excess of 3 meq H^+ did not exceed the proton-accepting capacity of the INT used in the mixture. Lower concentrations produced a linear relationship between the formazan produced and the proton supply. Thus, under the time, temperature, and pH regime used in these experiments, it is possible to express the enzyme activity observed as meq H^+transferred/g dry weight or organic weight/hour. When samples from natural populations are used, units of meq H^+transferred/m^3 sea water/hour would be appropriate.



ıre 11. Calibration curve of formazan development optical density *vs.* concentration of ascorbic acid where 1 ml 0.01 M ascorbic acid is equivalent to 0.01 meq. H+.

Activity in Biological Material. We can report only preliminary results from o marine organisms used in laboratory experiments. These were the atherinid nnow, *M. menidia*, and a decapod crustacean, *Palaemonetes vulgaris* (Say). Respiration rates of invertebrate animals vary with environmental conditions h as temperature and oxygen tension, and with species, size and activity. ough not a planktonic organism, *M. menidia* was used initially because it s easily obtained and weighed, and its tissues showed high activity. Oxygen ake by a single fish was measured for 30 minutes, after which its tissues were nogenized and the dehydrogenase activity measured (Fig. 12). There is a itive, linear relationship between oxygen consumption and enzyme activity. gression lines have been fitted to the experimental points by the method of t squares. The correlation coefficient (r) of 0.91 is significant at the 0.01 el. Neither regression line passes through the origin, although Y as a func- of X approaches it closely. These data indicate that a small amount of yme activity should be observed in the complete absence of gaseous respi- on. Rates of oxygen consumption above 0.4 ml/g/hr., as found in *M. menidia*, normally associated with active and vigorous swimmers such as mackeral *mber*) (Baldwin, 1924). The scatter of the points may be attributed to vary- size and activity of the specimens, although specimens averaging 40 mm e used.

Enzyme activity per unit weight of material as a function of specimen size also measured (Fig. 13). Activity decreased 50% with a ten-fold increase ody weight. A highly significant correlation coefficient of –0.99 has been

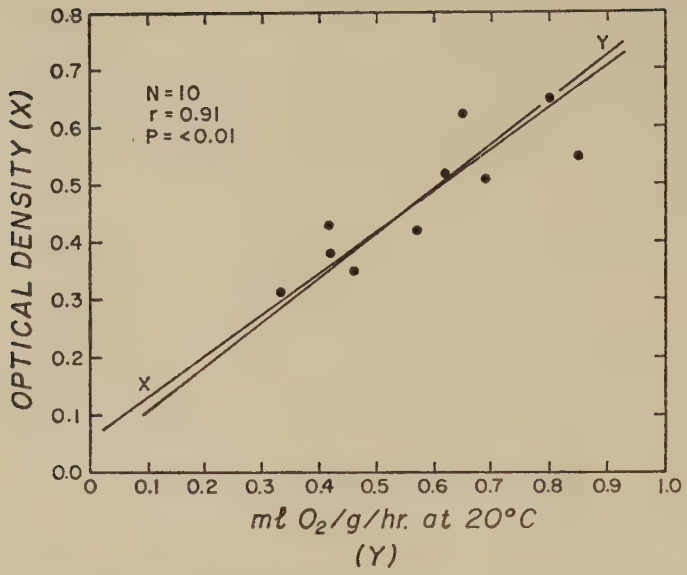


Figure 12. Oxygen consumption of *Menidia menidia* and equivalent formazan production of a homogenate containing 25 gm wet weight/ml of homogenate.

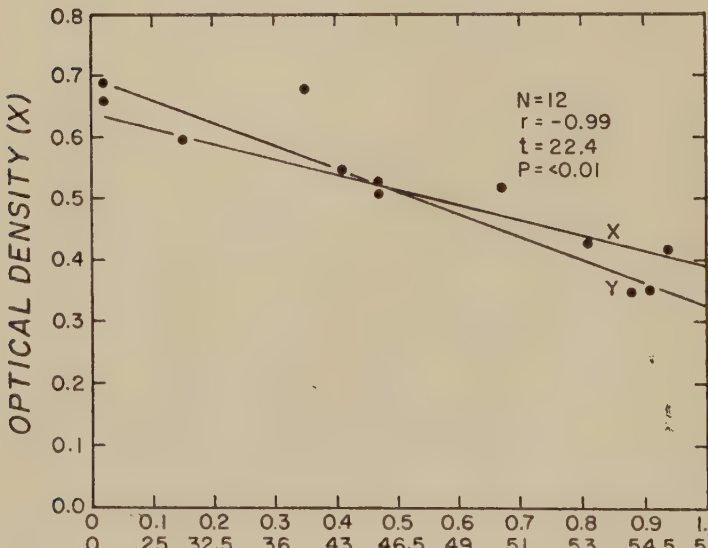


Figure 13. Formazan production per 25 mg wet weight of *Menidia* homogenate for various sizes of fish.

omputed for this relationship. Since activity is plotted as a function of body ize, the scatter in the data must be attributed to individual differences between pecimens and to experimental errors. The data were replotted as enzyme activity for a whole organism against body weight. Oxygen consumption was eplaced with formazan production in the formula:

$$cc\ O_2 = k\,W^x,$$

where k is a species constant, W is the wet weight of the organism, and x is deally 0.66 if oxygen consumption is exactly related to surface area, since surface area changes as the $^2/_3$ power of the weight. The formula:

$$O.D. = 0.133\,W^{0.7}$$

its the replotted data very closely. An exponent of $x = 0.7$ is a commonly eported value for *Menidia*-sized organisms (Zeuthen, 1953).

Several experiments were performed on freshly caught and starved fish. Starving for eight days decreased oxygen consumption by 40% and enzyme ctivity by 20% in 0.4–0.5 g specimens. Undoubtedly some of the observed epression of oxygen consumption is due to acclimatization to experimental onditions; depressed enzyme activity is probably a better indication of the hysiological condition of the specimens

DISCUSSION AND CONCLUSIONS

The method of measuring respiratory potential described here should be a seful one for both laboratory and field work. It has the advantage of being echnically uncomplicated and dispenses with the need of maintaining *in vivo* onditions during the measurement. Thus the technique, through the extraction of formazan, may be carried out on board a vessel, and the absorbance of he solution may be measured later, ashore. Care must be taken to prevent vaporation of the solvent and bleaching of the formazan.

The parameter being measured by our procedure has been called "respiratory otential" because the gross activity of an enzyme, which is unlimited by substrate concentration, should be constant per unit weight of enzyme. In effect, he amount of enzyme or, the capacity of the system, is being determined. This apacity or potential should be maximal with respect to the actual respiratory ctivity of the organism, which may depend upon food eaten, motor activity, emperature, etc. A linear relationship between gaseous respiration (oxygen consumption) and enzymatic activity (tetrazolium reduction) was found in *M. enidia*, indicating that measurements of dehydrogenase activity may be substituted for *in vivo* measurements of gaseous respiration. Preliminary work at ea indicates that all of the animals tested thus far (copepods, euphausids, ptero-

pods, chaetognaths, amphipods) possess a succinic dehydrogenase. However, evidence is not yet at hand to determine whether the same linear relationship that was found for *M. menidia* holds for these invertebrates.

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A Multipurpose Large Volume Sea-Water Sampler[1]

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The need for large volumes of sea-water, from all depths, for radioisotope udies with carbon-14, tritium or fission-products, has resulted in the develop- ient of a variety of sampling devices. Most of these appear to us to suffer from wo serious defects: one, they are clumsy to handle and often require large iameter wire for lowering; and two, they are special-purpose devices giving mples of little value except for a particular analysis. In fact, large volume mples from all depths are also needed for a variety of nonradiochemical pur- oses: trace element determinations, studies of organic matter, both dissolved nd particulate, nutrilite studies of phytoplankton, and others. The device de- cribed below has been developed and used with a view to satisfying the dis- arate requirements of all these programs.

Principle of Operation. This sampler is a modified Kammerer bottle, built f aluminum and stainless steel, Neoprene or Polyethylene valves, and lined vith Kel-F plastic. Models of 64 L and of 140 L capacity have been built. Their dimensions are identical except that the smaller has a 10″ diameter mple chamber. The drawings of the 140 L model in Fig. 1 will serve for escription of both models.

The sample chamber is an aluminum cylinder, 15″ O.D., and 57 3/4″ long; t each end a 45° neck leads to a six″ opening. The whole inside chamber, and he outside of the valve opening for about 1/2″ are spray-coated with Kel-F lastic (Trifluoro, chloro ethylene polymer). Lugs with 13/16″ diameter holes re welded to the outside of the chamber in three vertical rows, each consisting f three lugs; by means of these the chamber is slipped into position on three ails of 3/4″ diameter stainless steel rods. These rails are then bolted at each end o triangular aluminum plates, making a rigid frame. Between the top plate nd the sample chamber is the upper valve plate, which also slides freely on the ails. The sample chamber is connected to each corner of the upper valve plate

[1] Contribution No. 1208 from the Woods Hole Oceanographic Institution.

by 14 1/4″ lengths of chain. On the lower surface of the upper valve plate and on the upper surface of the bottom plate are neoprene and gum rubber valves, in the form of stoppers, which fit tightly in the 6″ openings of the sample chamber. On the upper surface of the upper valve plate is a catch held in the cocked position by the trigger of the release mechanism on the top plate. Thus, when cocked (Fig. 1 A), the sampler is open, the chamber hanging with its top opening about 7″ below the upper valve, and its bottom opening about 8″ above the bottom valve. When the messenger releases the trigger, both upper valve plate and sample chamber fall free; the inertia of each is sufficient to seat first the lower and then the upper valve, enclosing the sample to be collected.

Construction Details. Blueprints of engineering drawings of the sampler are available on request from the Woods Hole Oceanographic Institution; requests should be addressed to the third author.

Aluminum alloys of high corrosion resistance, like 6061-T6, should be used. It is also important that all moving parts be fitted very loosely, to allow accumulated salt particles to be flushed out easily.

Although handles, as shown in Fig. 1, are a real convenience, they have proved in practice to be quite vulnerable. One sampler, which has now been in use for two years with both handles gone, offers no serious problem in manipulation at sea, so these may be regarded as luxuries.

A small vent tube, 1/4″ I.D., as shown at the top of the sample chamber, is necessary to permit outflow of enough water to allow the valve to seat. This may be a standard one-way pressure relief valve or simply a short open tube; we have used both with no measurable difference in performance. The latter is somewhat more convenient as a point of attachment for an air pump to speed transfer of the water sample to shipping drums on deck.

The release mechanism, a stainless steel trigger (as shown in Fig. 1 A) slotted to permit the wire pennant to pass through it, is hinged on a pin at the bottom of the top plate. On the upper side of the top plate is a loosely attached trigger plate, through which the wire pennant is also passed. After passing through trigger plate, top plate and trigger, the wire is brought around a fixed semi-cylindrical bearing (3″ diameter), looped with a thimble over a 3/8″ aluminum bar on the upper side of the top plate, and fastened with wire clamps. In the cocked position, the trigger engages the U-shaped catch on the upper surface of the upper valve plate. To prevent pre-tripping due to vibration during lowering, it is necessary that the springs (bronze leaf springs) shown holding the trigger forward in the cocked position be relatively strong. To overcome their action, and the frictional resistance of the release, a brass messenger of five pounds weight is needed. The trigger plate simply transfers the weight of the messenger to the trigger, in spite of moderate angular displacement of the wire pennant relative to the sampler.

The upper and lower valves consist of five pieces each: distally, a large spacer of 1/16″ neoprene sheet; next a 1/2″ thick circle of natural rubber gum cemented to a 1/2″ thick circle of neoprene, shaped to fit the 6″ opening of the chamber; finally a Kel-F coated aluminum disk of 5 3/4″ diameter tapped to receive a 3/8″ diameter stainless set screw, which holds the valve to the surface of the plate (see Fig. 1 B) and which can be tightened to compress and expand the valve to compensate for wear in use. When all adjustments are properly made, the water sample comes in contact with only the 6″ diameter ring of neoprene (about 1/4″ wide), left uncovered by the Kel-F coated disk; the foam rubber contacts only the sides of the chamber opening.

The upper valve plate is weighted with three lead cylinders, each of five pounds. This has proved ample mass to seat the valve securely.

The Kel-F coating is applied by a standard commercial process licensed by the M. W. Kellogg Company. A directory of firms equipped to apply this material is obtainable from the Company.

Operation. In Fig. 2 is presented a block diagram of the rigging of the sampler on hydrographic wire. The six-foot pennant of the sampler is fastened through a heavy duty (at least 600 lbs. load) ball-bearing swivel to the end of the hydrographic wire. About six feet above the swivel is rigged a Nansen bottle with both protected and unprotected reversing thermometers. The sampler messenger, fitted with an arm long enough to by-pass the swivel, is hung by a light wire pennant from the Nansen bottle messenger-release mechanism. Tripping at depth is thus managed by first tripping the Nansen bottle, which then trips the sampler. The sample depth is determined from the thermometer readings, and proper operation of the sampler is determined by comparison of salinity between the sampler contents and that of the Nansen bottle.

Aside from the usual spectrum of ills to which a lowered hydrographic device is subject, two other problems are inherent in the design of this sampler:

A surge of the sea surface which meets the sampler when lowered will not fill the chamber fast enough to prevent its floating. In extreme cases this may trip the release—defining the sea-state beyond which work is no longer possible. In less than extreme conditions, the chamber may float up and seat the upper valve firmly enough to suspend the chamber from it; if not noticed, this results in lowering a bell closed at the top and in collecting a sample of surface water only slightly mixed with water from below. This can be completely prevented by clamping to one or two rails, just above the position of the lower lug when cocked, a stop which will not permit the barrel to rise enough to reach the upper valve in the cocked position.

Because of the relatively low mass of the sampler and its high cross-sectional and surface area, the sampler must not be lowered at too high winch speed, otherwise the wire will finally pass the sampler. Using 3/16″ stainless wire, and the 140 L sampler illustrated herewith, a maximum speed of lowering of about

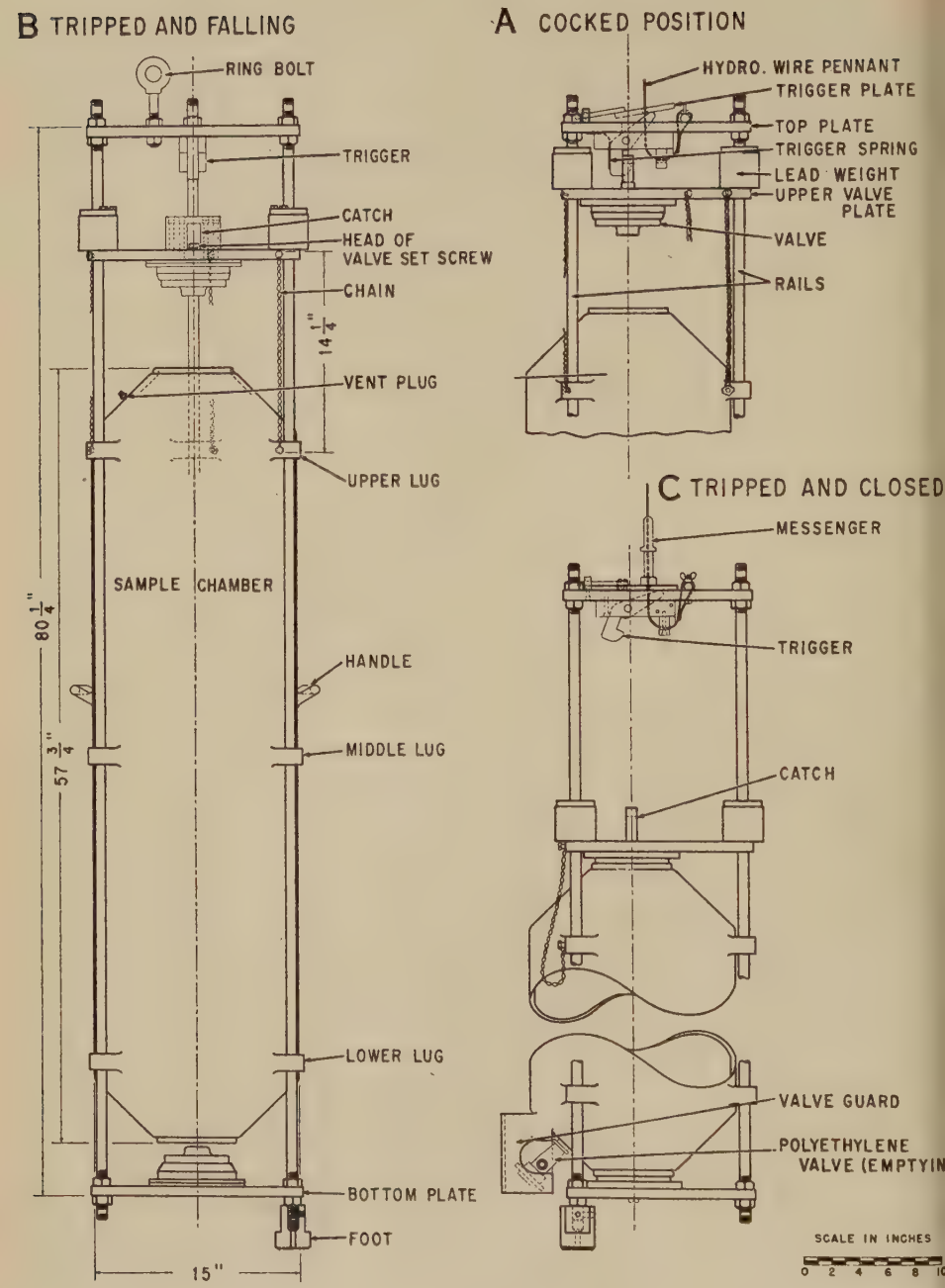
B TRIPPED AND FALLING
RING BOLT
TRIGGER
CATCH
HEAD OF VALVE SET SCREW
CHAIN
14 1/4"
VENT PLUG
UPPER LUG
SAMPLE CHAMBER
80 1/4"
57 3/4"
HANDLE
MIDDLE LUG
LOWER LUG
BOTTOM PLATE
FOOT
15"
A COCKED POSITION
HYDRO. WIRE PENNANT
TRIGGER PLATE
TOP PLATE
TRIGGER SPRING
LEAD WEIGHT
UPPER VALVE PLATE
VALVE
RAILS
C TRIPPED AND CLOSED
MESSENGER
TRIGGER
CATCH
VALVE GUARD
POLYETHYLENE VALVE (EMPTYIN
SCALE IN INCHES
0 2 4 6 8

Figure 1.

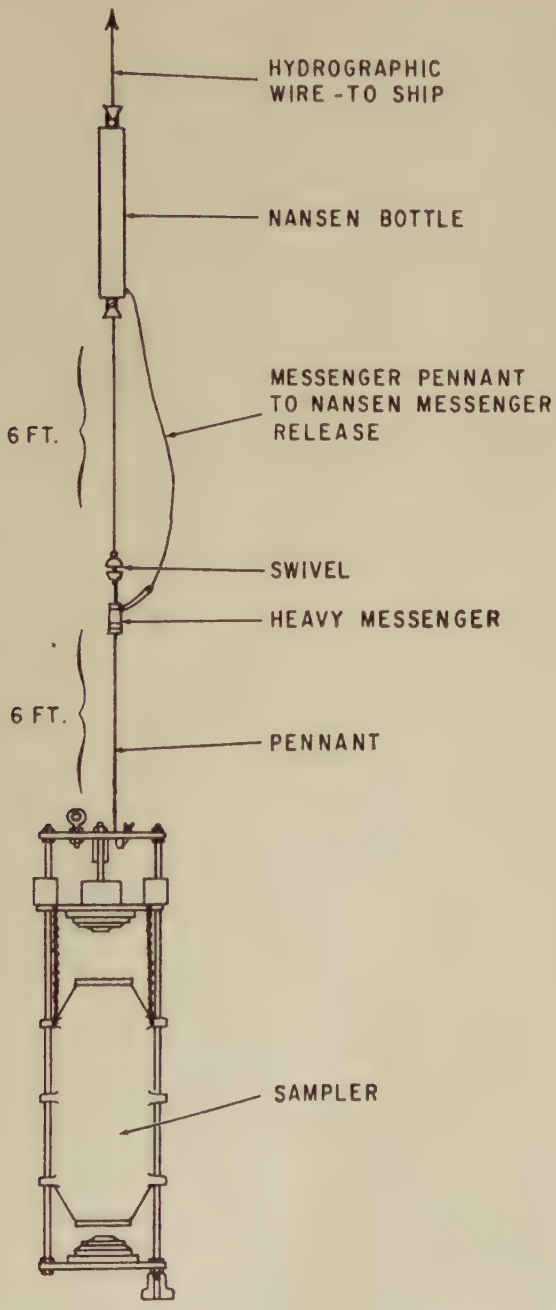
HYDROGRAPHIC
WIRE - TO SHIP
NANSEN BOTTLE
MESSENGER PENNANT
TO NANSEN MESSENGER
RELEASE
6 FT.
SWIVEL
HEAVY MESSENGER
6 FT.
PENNANT
SAMPLER

Figure 2.

45 m/min. has been found to be safe. The full sampler can be raised at normal operating maximum speed.

During the operation of getting the cocked sampler over the side, considerable danger—in fact, substantial certainty—of a pre-trip exists. This is obviated by placing a large clamp on one rail to hold the upper valve plate in cocked position. After resetting the trigger if necessary, and after the Nansen bottle has been rigged, this clamp is removed just before lowering away.

No general prescription for ship handling can be made. The three devices now in use have seen service from RV PANULIRUS (50 feet) of the Bermuda Biological Station, and from RV BEAR (103 feet), RV CRAWFORD (125 feet) and RV CHAIN (215 feet) of the Woods Hole Oceanographic Institution fleet. In each case the details of rigging have presented new, but solvable, problems. Both 5/32 and 3/16 wire have been used; so far no sampler has been lost.

The stations for which these samplers are used normally require from 6–11 lowerings, and the time to empty the chamber through the 1/2″ valve used is about 15 minutes. Thus the saving in ship time by having two samplers in use alternately rather soon pays for the second device. Operating in this way, a station with samples at surface, 100, 300, 500, 700, 1000, 1500, 2500, and 4000 m can be obtained in less than 10 hours.

Performance Data. Table I compares hydrographic data from a recent station made with the large volume sampler, with data from a regular hydrographic station made with Nansen bottles in series just before the large sampler station. In general the salinities of sampler and simultaneous Nansen bottle agree within the precision of the salinometer employed (Schleicher and Bradshaw, 1956). It is seen that the lowerings to 91 and to 297 m spanned regions of rapid change in salinity; in both cases the flushing of the large sampler has been thorough enough to show the difference in salinity over the approximately 5 m difference in depth (Fig. 2) between it and the Nansen bottle. This confirmation of the excellence of flushing, at least by comparison with the Nansen bottle, has been the usual observation whenever sharp gradients have been encountered.

So far these large samples have been used principally for radiochemical studies (Bowen and Sugihara, 1960), with complete satisfaction. Unpublished studies by Curl and Bowen have indicated they are also suitable for separation of organic particulate matter; others by Ryther have shown that the water is suitable for culture experiments with marine phytoplankton. We believe that, with slight modification, the samples would prove suitable for trace element analysis of both dissolved and particulate phases; as now used, the sample chamber is painted outside with a zinc chromate anti-corrosion paint. Although the filtered material from these samples showed less paint contamination than is usual from either Nansen or Van Dorn bottle samples, we believe trace element samples should be collected with an unpainted and probably freshly polished device.

TABLE I.

Cruise CHAIN 17 — Station Number 341
Position: 00°13′S; 18°34′W — Date: 24-IV-61

Hydrographic Station			Large Sampler Station			
Corr. Depth (m)	Temp. (°C)	Salin. (‰)	Corr. Depth (m)	Nansen Temp. (°C)	Nansen Salin. (‰)	Sampler Salin. (‰)
1	29.27	35.350	1	–	–	35.008
30	27.13	36.051				
60	20.42	36.241				
99	16.05	35.704	91	15.16	35.596	35.526
149	14.22	35.443				
199	13.21	35.298				
248	12.13	35.170				
298	10.54	34.975	297	10.29	34.954	34.939
398	8.56	34.757				
			494	6.73	34.592	34.590
596	5.47	34.504				
			692	5.16	34.496	34.500
795	4.55	34.495				
994	4.39	34.612	987	4.39	34.616	34.616
1193	4.42	34.786				
1392	4.31	34.924				
			1494	4.07	34.966	34.961
1587	3.98	34.960				
1887	3.46	34.958				
2187	3.298	34.952				
2487	2.971	34.929	2485	2.961	34.933	34.930
2787	2.730	34.916				
3087	2.607	34.903				
3487	2.479	34.896				
3887	2.226	34.879				
4287	1.334	34.778				
4687	1.092	34.749				
5087	1.110	34.746	5003	1.120	34.758	34.754
5378	–	34.754				
5578	1.150	34.750				
5778	1.185	34.748				
6076	1.231	34.748				
6374	1.274	34.753				
6673	1.317	34.748				
			6774	1.33	34.748	34.899
6872	1.344	34.750				
7072	1.377	34.750	7072	1.41	34.750	34.753
7271	1.407	34.746				
7570	1.451	34.746				

Because of the large volume of sample obtained, very little change of temperature occurs during retrieval. Although no advantage has been taken of this fact so far, the samples would be very suitable for collection of living organisms, especially low-temperature stenotherms.

Acknowledgments. It is a pleasure to thank our many co-workers who have assisted in this development, both in making useful design suggestions and in working out safe procedures for ship handling. We are most grateful to personnel of the American Durafilm Company, Newton Lower Falls, Massachusetts, for assistance in technical aspects of the plastic coating. The original development work was supported very largely by the National Science Foundation — International Geophysical Year program. Additional support has been received from the Office of Naval Research and from the Atomic Energy Commission under grant AT(30–1)–2174.

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